JOURNAL OF DAIRY SCIENCE

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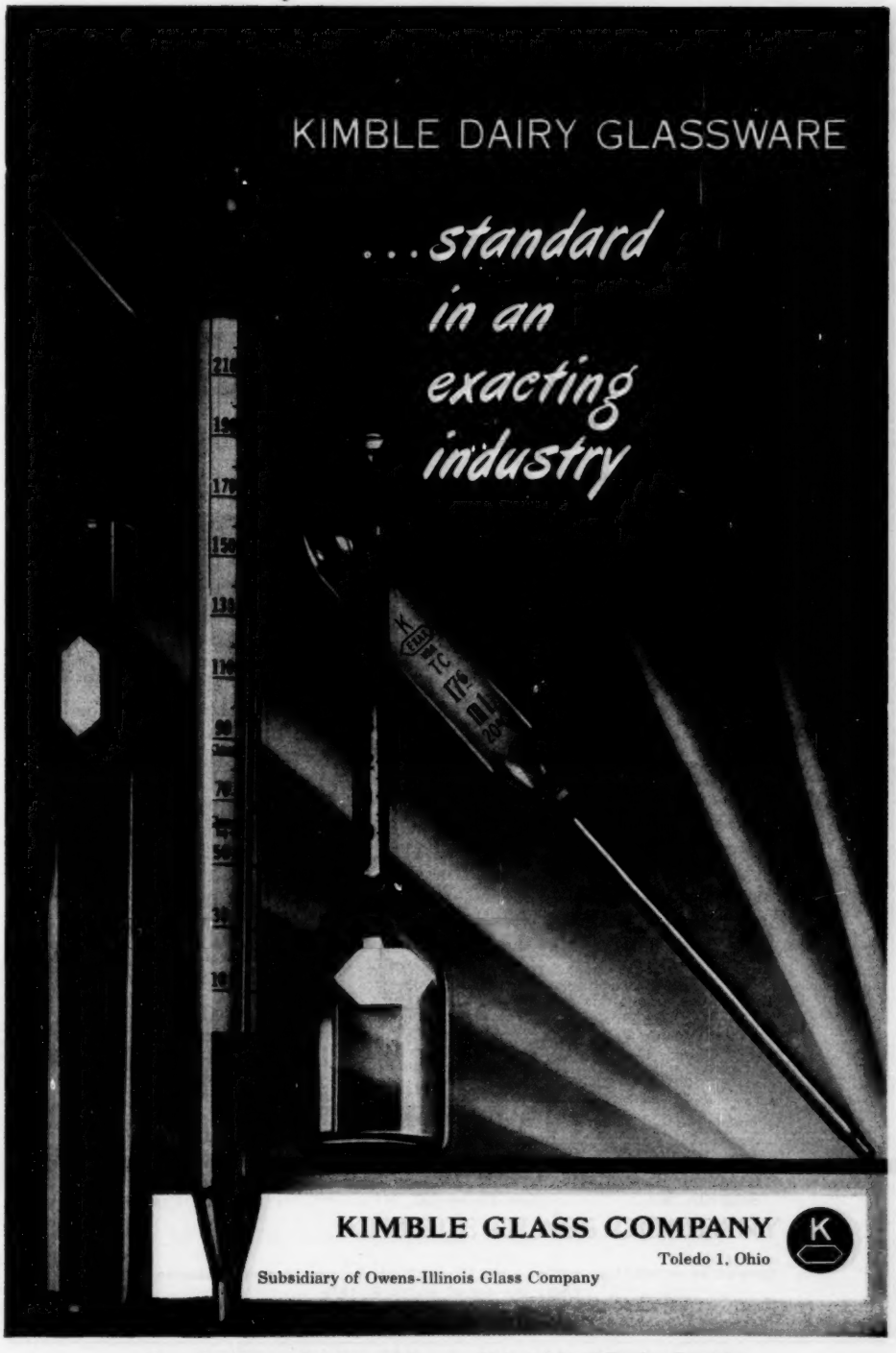

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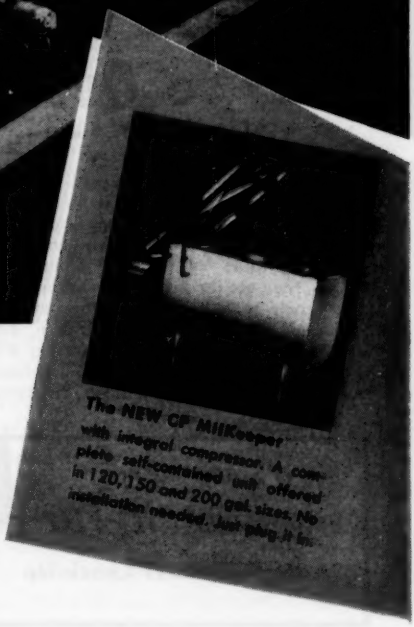

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VOLUME XXXV NOVEMBER, 1952 NUMBER 11

FACTORS INFLUENCING THE AMOUNT OF MILK "LET-DOWN" HORMONE IN THE POSTERIOR LOBE OF JERSEY CATTLE[1]

W. G. WHITTLESTONE, E. G. BASSETT AND C. W. TURNER[2]

Ruakura Animal Research Station, Hamilton, New Zealand, and the Missouri Agricultural Experiment Station, Columbia

The presence of a factor in the posterior pituitary which would stimulate lactating animals to make their milk available was discovered by Ott and Scott in 1910 (4). Evidence that the factor caused the contraction of the gland rather than the stimulation of milk secretion soon was forthcoming (1, 2, 5, 6).

The nature of the hormone has received considerable study. With the separation of the posterior lobe principles into two fractions, one predominately oxytocic and the other vasopressor and antidiuretic (3), the question of the nature of the milk "let-down" factor was raised. Turner and Cooper (7) developed an assay technique using lactating rabbits. They observed that both pitocin (oxytocic factor) and vasopressin (vascular factor) stimulated milk let-down to a greater extent than could be caused by the contamination of the corresponding hormone. It was suggested that a specific mammary gland stimulating hormone might be present in the posterior lobe.

Recently, Whittlestone (9) developed an assay technique for milk let-down substances using the time during which milk can be removed from the glands of a lactating sow as the index of response. It was shown that the oxytocic as well as the vasopressor fractions of commercial preparations produced milk let-down, although the former was far more potent than the latter on a unitary basis. Five units of a highly purified vasopressor fraction showed the same milk let-down activity as one unit of standard oxytocin (9).

From these observations, it appeared evident that only by the assay of the posterior lobe, using the milk let-down phenomenon as an index of biologic activity, could the true picture of its capacity to cause milk let-down be assessed.

In a preliminary study of the neurohypophysis, Whittlestone *et al.* (10) assayed separately the median eminence of the hypothalamus, the stalk and the posterior lobe. It was shown that the median eminence usually contains an insufficient amount of hormone to give even a minimal response in the sow. The stalk, however, contained, on a dry matter basis, about one-tenth or less the amount of hormone of the posterior lobe.

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[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1306.

[2] Fulbright Research Scholar.

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The present paper for the first time presents data on the amount of milk let-down hormone in the posterior lobe of a series of calves of increasing age, of heifers and of lactating cows. The pituitaries of identical twin heifers and cows are included. The effect of the time after milking upon the hormone content is noted.

EXPERIMENTAL

Within 30 min. after the animals were killed, the entire neurohypophysis was dissected out, dropped into cold acetone and stored at – 12° C. The next day the median eminence, the stalk and the posterior lobe were separated, further defatted and dehydrated in changes of acetone, dried and weighed. The milk

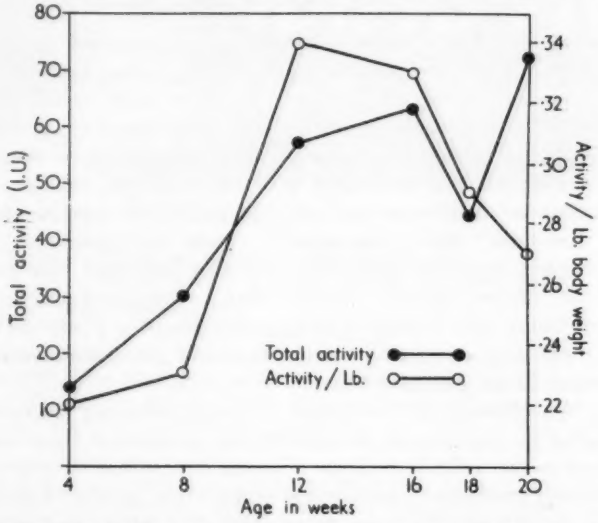

FIG. 1. Trends in the total milk let-down activity and the activity per unit body weight of the posterior lobes of Jersey calves.

let-down hormone was extracted and assayed by methods previously described by Whittlestone (9). The amounts of hormone in the stalk and in the posterior lobe were assayed separately in terms of standard oxytocic hormone.

RESULTS

The pituitaries of grade Jersey calves at 4 wk. of age contained from 14 to 22 I. U. of hormone and from 0.17 to 0.24 I. U. per pound of body weight. With increasing age and body weight at 2, 3, 4 and 5 mo. the amount of hormone present increased per gland in the young animals (fig. 1). The older cows appear to have less hormone than the 5-mo.-old calves. The milk let-down activity per unit of body weight increases in the calf until at 3 mo. it seems to remain fairly constant. However, the mature cows have very much less activity on a body-weight basis. The amount of hormone per pituitary was 108 I. U. in a 5-mo.-old

calf and the greatest concentration per pound body weight was 0.59 I. U. in a 4.5-mo.-old calf (table 1).

A single set of identical twin heifers, T17 and T18, about 15 mo. of age was available for study. T17 was 3 mo. pregnant. She showed the highest amount

TABLE 1

The milk let-down hormone content of the posterior pituitary of growing, pregnant and lactating Jersey cattle[a]

No.	Age	Body wt.	Posterior lobe			Activity/lb. body wt.	Relative wt. of post. lobe	Notes
			Wt.	Total activity	Specific activity			
	(*wk.*)	(*lb.*)	(*mg.*)	(*I. U.*)	(*I.U/mg.*)		(*mg./lb. body wt.*)	
Foetus	12	.....	90	None	.....	.....	.....	
102	4	81	129[b]	14	.....	0.17	.....	
104	4	82	116[b]	20	.....	0.24	.....	Male calf
12	4	93	20	22	1.1	0.24	0.22	
105	8	113	Lost	.....	.....	.....	.....	
106	8	123	31	31	1.0	0.25	0.25	
11	8	132	25	28	1.1	0.21	0.19	
32	12	190	31	59	1.9	0.31	0.16	
30	12	148	22	55	2.5	0.37	0.15	
10	12	187	26	16	0.6	.....	.....	Posterior lobe incompletely separated
107	16	207	32	74	2.3	0.36	0.16	
101	16	182	26	52	2.0	0.29	0.14	
1	18	156	26	23	0.9	0.15	0.17	
2	18	205	49	49	1.0	0.24	0.24	
3	18	175	25	28	1.1	0.16	0.14	
18	18	127	29	75	2.6	0.59	0.23	
100	20	251	60	108	1.8	0.43	0.24	
33	20	301	33	58	1.8	0.19	0.11	
26	20	291	32	51	1.6	0.18	0.11	
17	64	560	71	178	2.5	0.32	0.13	Pregnant (Foetus pituitary assayed)
18	64	460	44	79	1.8	0.17	0.10	
	(*Yr.*)							
608	2	662	57	37	0.6	0.056	0.09	Milked 5 hr. before slaughter
73	3	580	48	23	0.5	0.040	0.08	Milked just before slaughter
89	3	597	59	22	0.4	0.037	0.10	Milked 5 hr. before slaughter
598	3	763	82	31	0.4	0.041	0.11	Milked 19 hr. before slaughter
563	5	708	23	13	0.6	0.018	0.03	Milked 6 hr. before slaughter
T5	5	797	79	40	0.5	0.050	0.10	Identical twins
T6	5	756	83	58	0.7	0.077	0.11	Identical twins
J45	5	1009	41	21	0.5	0.021	0.04	Identical twins; Ayrshires
J48	5	1026	95	38	0.4	0.037	0.09	Identical twins; Ayrshires

[a] All animals except J45 and J48 were grade Jerseys.
[b] Whole pituitary weighed and assayed.

of hormone per pituitary of any animal studied with 178 I. U. Her non-pregnant mate's gland contained less than half this amount. These data suggest that pregnancy may have a marked effect on the amount of milk let-down hormone, but further observations on identical twins are required.

The pituitaries of nine cows in varying stages of lactation were assayed.

Some of these cows were killed before being milked, whereas others were sacrificed shortly after being milked. It is apparent that the amount of let-down hormone released at milking is not sufficient to exhaust the gland. In the case of cow 73, milking with a normal let-down was carried out only a few minutes before slaughter. The posterior lobe still contained 23 units, which would be much more than required to effect another release of milk. The amount of hormone required for a normal let-down seems to be less than the variation in the amount of the substance present in the posterior lobes of different animals. These observations support the view previously expressed by one of the authors (8) that the cow is capable of more than one release of let-down hormone at a milking.

Special interest is attached to the observations on two sets of identical twin cows, T5 and T6 and J45 and J48. The twins show little similarity in the amount of hormone present. In the case of twins J45 and J48, even the dry weights of the posterior lobes are markedly different. The ratio of the weight of the dry defatted posterior lobe to the animal's body weight in general seems to be erratic. In the case of the calves it shows no trend in the period from 1 to 5 mo. While twins 5 and 6 show a similar figure, twins J45 and J48 are quite dissimilar.

J45 and J48 were 5-yr.-old Jersey-Ayrshire cows in full lactation. While they had large udders, their milking characteristics were difficult and their lactation periods were short. When killed, these cows had been nursing calves which were separated from them at 5 p. m. the previous day. The time interval was 16 hr. for J48 and 18 hr. for J45. From the point of view of the interval between let-down and slaughter these cows are comparable with cow 598. Any generalization concerning the relationship between ease of milking and the amount of let-down hormone is not possible because all of the cows were culls removed from the herd because of unsatisfactory production. In several cases, *e.g.*, twins 5 and 6, this was in large degree due to milking difficulty and it is likely that this factor was in part involved in the case of the others.

SUMMARY

The milk let-down hormone content of the posterior pituitary glands of dairy cattle has been estimated. The cattle ranged in ages from 1 mo. to 5 yr. Eighteen calves between the ages of 1 and 5 mo., two 15-mo. old heifers and five cows ranging from 2 to 5 yr. were included in the study. The weight of the dried, defatted posterior lobe, its total milk let-down activity, activity per unit weight of dried gland and the activity per unit of body weight were determined. The total milk let-down activity rises with age in calves, while the mature cows show a decline. The activity per unit of body weight rises in calves until at about 3 mo. the figure reaches a fairly steady level. Mature cows show a much lower relative level. The weight of dried defatted posterior lobe shows no trends with increasing age in calves but appears to fall in mature cows.

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FREQUENCY OF DISCHARGE OF PITUITARY MILK "LET-DOWN" HORMONE[1]

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Cows normally are milked at 8- to 14-hr. intervals. At milking time the let-down of milk generally is thought to be caused by the discharge of "let-down" hormone from the posterior pituitary, causing a contraction of the mammary myoepithelium.

Many years ago at the Missouri Station, the problem of the rate of milk secretion during the interval between milkings was under investigation (2, 3, 5). An attempt was made to remove the milk at 1- or 2-hr. intervals throughout the day and thus determine the average hourly rate of milk secretion. When cows were milked at hourly intervals, however, usually only a small amount of milk was removable until, after 3 to 5 hr., the cow would let down her milk and a larger yield was obtained. Subsequent hourly milkings again were ineffective in removing the milk believed to be present until several hours had passed. Similar results were obtained by Filipovic (1) and more recently by Smith (4). These observations led Turner to the view that when the pituitary had been stimulated to discharge a normal quantity of the let-down hormone, a number of hours was required for the gland to secrete enough let-down hormone to permit another release of milk.

Recently, Whittlestone (6) reported that some cows will give one-half to two-thirds of their milk to the machine but only yield the remainder after a considerable amount of vigorous machine stripping accompanied by udder massage or after normal hand stripping. Following this treatment, the cow yielded a "second flow of milk". Experiments were described which supported the view that in such cases the cow does actually respond to the stimulation with a further release of pituitary milk let-down hormone.

If cows are capable of giving a second release of let-down hormone within a period of 20 min., as suggested by Whittlestone, the earlier failure to obtain a full yield of milk after periods of 1 or 2 hr. obviously requires further study. The present report suggests an explanation of the earlier results and presents data indicating that the full yield of milk secreted during a 2-hr. period can be obtained at a normal milking.

EXPERIMENTAL

In initiating these experiments it was believed to be important that the regular milker conduct the tests, so that the cows would react normally. The

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[2] Fulbright Research Scholar.

only variation from normal was bringing the cows in from the pasture every 2 hr. during the day. Preceding the application of the teat cups, the teats were washed and the udder massaged. The strip cup then was used. This preparation occupied about 1 min. The rate of milk removal in the second and third experiments was recorded automatically (figs. 1 and 2).

In a preliminary trial, a cow with a good temperament was selected. She responded rather uniformly, except at one period when a visitor to the shed disturbed her. It then was decided to use a set of identical twins for the further

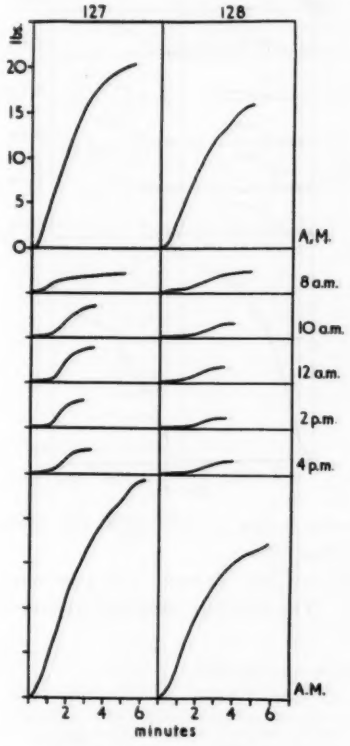

FIG. 1. Milk removal curves for twin cows milked at 2-hr. intervals throughout the day. The two uppermost curves were recorded at the morning milking of the day of the experiment. The bottom curves were recorded at the morning milkings on the day following the experiment.

tests. They were milked at 2-hr. intervals during the day. In the second of these trials, cow no. T.127 was given 30 I. U. of pitocin and remilked immediately after the morning milking to remove the residual milk. The samples of milk were tested for fat content, total solids and solids-not-fat.

RESULTS

It will be seen in the case of cow T.205 (table 1) in the preliminary experiment that milk let-down occurred following stimulation and the milk secreted during the 2-hr. interval was obtained. Unfortunately, at the noon milking, a visitor came to the cow shed and only a partial let-down occurred. This re-

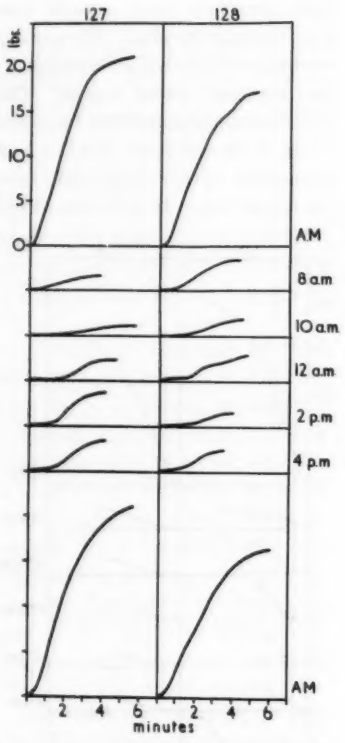

FIG. 2. Milk removal curves for twin cows milked at 2-hr. intervals. Following the normal morning milking shown by the uppermost curves, cow T.127 was given a subcutaneous injection of 30 units of oxytocin and remilked.

sulted in a larger yield at the 2 p.m. milking and a return to normal at the final period. The fat tests were either about normal or above.

Considering the rate of milk secretion of this cow, the average rate per hour was 2.3 lb. or 4.6 lb. per 2-hr. milking interval. The amount obtained at the 8

TABLE 1

Milk yield and composition obtained at 2-hr. intervals

(Experiment I. Sept. 16, 1951. Cow no. T.205)

Time	Milk yield	Fat	Total solids	Solids-not-fat	Solids-not-fat (fat-free basis)
	(*lb.*)	(%)	(%)	(%)	(%)
4 p.m. (15th)	19.5	5.30	16.33	9.03	9.53
6 a.m.	18.9	2.33	11.69	9.36	9.58
8: 05 a.m.	4.3	5.95	14.74	8.97	9.54
9: 55 a.m.	4.8	7.40	16.18	8.78	9.49
11: 55 a.m.	2.0[a]	5.60	14.49	8.89	9.41
1: 55 p.m.	8.4	8.23	17.03	8.80	9.59
3: 55 p.m.	3.6	5.98	14.69	8.71	9.27
Total	23.1				

[a] Cow disturbed by visitor to the cowshed.

and 10 a.m. periods are very close to the average. The total for the next 4 hr., 8.4 lb. or 4.2 lb. per 2 hr., also closely approaches the average rate. The total yield for the previous 24 hr. was 28.4 lb. or 3.2 lb. per 2-hr. period. Since this cow was milked at 10- and 14-hr. intervals, the accumulation of milk in the udder apparently tended to depress the rate of milk secretion. Obviously this cow reacted normally to the stimulus of milking by a discharge of the milk let-down hormone at 2-hr. intervals during the day except when disturbed. Toward the end of the day, the teats of the cow became somewhat tender, due in part to the

TABLE 2

Response of identical twin cows to milking at 2-hr. intervals

Time		Cow T.127					Cow T.128				
		Milk yield	Fat	Total solids	Solids-not-fat	Solids-not-fat (fat-free)	Milk yield	Fat	Total solids	Solids-not-fat	Solids-not fat (fat-free)
		(*lb.*)	(%)	(%)	(%)	(%)	(*lb.*)	(%)	(%)	(%)	(%)
10/10/51	4 p.m.	15.7	.....	.....	.....	.....	9.8	.....	.....	.....	.....
11/10/51	6 a.m.	20.3	3.4	12.01	8.61	8.91	15.8	3.38	13.69	9.31	9.63
	8 a.m.	2.4	9.95	17.59	7.64	8.48	2.5	9.30	17.86	8.56	9.44
	10 a.m.	3.4	6.15	14.73	8.58	9.14	2.0	6.13	15.13	9.00	9.59
	12 noon	4.1	8.13	16.49	8.36	9.10	1.7	6.05	14.98	8.93	9.48
	2 p.m.	3.3	5.85	14.46	8.61	9.14	1.5	5.85	15.07	9.22	9.78
	4 p.m.	3.1	6.18	14.71	8.53	9.09	2.0	6.25	15.22	8.97	9.57
	Total	16.3	.....	.....	.....	.....	9.7	.....	.....	.....	.....
12/10/51	6 a.m.	24.3	2.95	11.70	8.75	9.02	17.4	2.48	11.68	9.20	9.44
18/11/51	4 p.m.	15.7	4.10	12.80	8.70	9.07	11.1	4.80	13.93	9.13	9.58
19/11/51	6 a.m.	21.2	4.40	13.30	8.90	9.31	17.0	4.20	13.34	9.14	9.54
	Residual[a] Milk	1.3	10.71	18.94	8.23	9.21	.....	.....	.....	.....	.....
	8 a.m.	1.4	6.48	14.31	7.83	8.37	3.2	7.85	16.58	8.73	9.47
	10 a.m.	1.3	3.75	12.10	8.35	8.67	1.8[b]	5.90	14.97	9.07	9.64
	12 noon	2.5	3.40	12.66	9.26	9.58	2.5	5.63	14.87	9.24	9.73
	2 p.m.	3.6	5.50	14.39	8.89	9.41	1.4[b]	5.75	14.96	9.21	9.77
	4 p.m.	3.5	5.10	14.08	8.98	9.46	2.7	4.90	14.18	9.28	9.76
	Total	12.3	.....	.....	.....	.....	11.6	.....	.....	.....	.....
20/11/51	6 a.m.	21.2	3.65	12.66	9.01	9.35	16.5	3.75	12.79	9.04	9.39

[a] 30 I. U. of pitocin injected and cow remilked.
[b] Cow T.128 was annoyed by flies at these milkings.

lack of an end-point of the completion of milking, with the result that the teat-cups remained on the cow slightly longer than necessary. This was taken into consideration in the following trials.

In the study in which the twin cows served as experimental animals, not only does the fairly regular yield of milk at 2-hr. intervals indicate the induction of milk let-down but the similarity in the milk let-down curves for each animal is further evidence (figs. 1 and 2). The yield of milk obtained at 2-hr. intervals generally is fairly uniform (table 2). This indicates that the discharge of the let-down hormone had been induced in these cows by the stimulus of milking.

This is further confirmed by an examination of the curves of milk removal. At the regular milking period, the time lag between placing the teat-cups on the cows and the start of milk removal is very short. At the 2-hr. milking periods the interval is considerably longer but the characteristic curve of milk removal for each cow indicates the occurrence of let-down. The delay in let-down probably is due to the fact that the effectiveness of the milking stimuli is reduced when there is little milk in the udder.

In the case of cow T.127 in the second experiment with twins, the injection of 30 I. U. of pitocin caused the removal of 1.3 lb. of additional milk; the two subsequent periods yielded less milk than the average. Possibly this excessive amount of pitocin either influences the rate of milk secretion or the reduced yield at the subsequent milkings is due to the filling of the lumen of the alveoli with milk which normally constitutes part of the residual milk, *i.e.*, milk not normally obtainable by the discharge of let-down hormone.

SUMMARY AND CONCLUSIONS

Experiments are described which show that some dairy cows may be normally milked at 2-hr. intervals. By maintaining a normal milking routine at which only the regular milker was present it was shown that usual let-down and milk removal was obtained in three cows.

In the light of these results it seems likely that the previously reported failures when 2-hr. milking was attempted could be explained in terms of such an unusual procedure being associated with stimuli which inhibited the cows.

The results add further strength to the view that when incomplete let-down occurs the cow concerned may be persuaded by gentle massage of the udder and other similar stimulation to discharge a second quantity of let-down hormone.

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FERTILITY OF BULL SEMEN AS INFLUENCED BY DILUTION LEVEL, ANTIBIOTICS, SPERMATOZOAN NUMBERS AND THE INTERACTION OF THESE FACTORS

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Investigations by Salisbury and Bratton (7) and Willett (11) showed that as dilution levels of bull semen were increased above 1 : 100, the fertility of semen decreased. The latter work indicated, also, that as spermatozoan numbers were reduced below 6 million, the drop in breeding efficiency was more rapid than with numbers above this figure. In both these papers there was some evidence that the drop in fertility with increase in dilution level was less when sulfanilamide was in the yolk-citrate extender than when it was not. However, most of the data from these two studies were derived from samples containing sulfanilamide. Since that time, penicillin and streptomycin have been widely adopted as additional ingredients. The question then arose as to whether the decline in fertility with increasing dilution level was the same with as without antibiotics, and if the experimental results obtained before antibiotics were used still apply when antibiotics are included in extenders. Various workers (3, 4, 5, 6, 9, 11) have reported investigations of the direct, detrimental effect that high dilutions appear to have upon the physiological processes of spermatozoa.

This paper reports two experiments designed (a) to determine whether there is a difference in rate of decline in fertility with increase in dilution level when antibiotics are in semen diluter and when they are not, (b) to measure the relative importance of dilution effect and spermatozoan numbers upon fertility of semen with dilution levels to 1 : 300 and (c) to give some indication of the fertility of semen containing varying and low numbers of spermatozoa. An incidental study of field data to supplement this third phase also is reported.

ANTIBIOTICS AND DILUTION LEVEL

In the two experiments, a collection of semen consisting of two or more ejaculates from one bull mixed together was divided into four parts. Two parts were diluted 1 : 100 and the other two 1 : 300 in yolk-citrate-sulfanilamide. To one of the parts at each dilution level were added sodium penicillin G and dihydrostreptomycin sulphate and to the other none. The concentration of these antibiotics was 1,000 units or micrograms per milliliter in the first experiment and 500 in the second. Other work (10) has indicated that one can expect no difference in breeding efficiency between semen containing these two different concentrations. Sodium citrate dihydrate and sulfanilamide were used in the buffer at the rates of 2.9 and 0.6 per cent, respectively. The proportion of yolk and buffer was 1 : 1. With successive collections these four treatments were rotated among four inseminator groups. Four collections, therefore, comprised a Latin

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square, and each experiment consisted of four squares. In experiment I, sixteen collections from six bulls (each used one to six times) comprised the four squares; in experiment II, each square represented one bull. Breeding efficiency was measured by 60- to 90-day non-returns to first services made the day following collection. In the first experiment, the semen was used in the field about 25 to

TABLE 1

Average non-returns with semen diluted 1:100 and 1:300 in yolk-citrate-sulfanilamide with and without penicillin and streptomycin

Expt. no.		Sulfa-penicillin-streptomycin			Sulfa only		
		1:100	1:300	Diff.	1:100	1:300	Diff.
I	Services (no.)	846	886		851	814	
	Non-returns (%)	71.6	61.2	10.4	70.3	61.3	9.0
II	Services (no.)	802	701		805	702	
	Non-returns (%)	67.4	61.3	6.1	64.7	59.4	5.3
Both	Services (no.)	1,648	1,587		1,656	1,516	
	Non-returns (%)	69.6	61.2	8.4	67.6	60.4	7.2

37 hr. and in the second, 30 to 42 hr. after collection. Number of services per sample varied from 11 to 159, with 22 of the 128 having less than 30.

In experiments I and II, the average spermatozoan concentration of the fresh semen was 1,182 and 1,168 million per milliliter, and range of sperm concentration 467 to 2,171 and 681 to 2,096 million, respectively. In this paper spermatozoan concentration or numbers refer to the total number of spermatozoa, dead and alive. The first experiment was conducted during July, August and September and the second during September, October and November of the following year.

TABLE 2

Average non-returns for antibiotic treatments and for 2 dilution levels (2 experiments)

Treatment	Services	Non-returns
	(*no.*)	(%)
1:100	3,304	68.6
1:300	3,103	60.8
Difference		7.8
Sulfa-penicillin-streptomycin	3,235	65.5
Sulfa only	3,172	64.2
Difference		1.3

Spermatozoan concentration was determined with a photelometer (12). All statistical analyses in this paper were in accordance with methods outlined by Snedecor (8).

Results and discussion. Summaries of results are presented in tables 1 and 2. In the data for the two experiments combined, the difference of 7.8 in non-return rate between dilution levels is very highly significant ($P < 0.001$) and the differ-

ence of 1.3 between antibiotic treatments is significant ($P < 0.05$). As can be seen in table 1, there was a slightly greater difference between dilution levels with antibiotics (8.4) than without (7.2). The difference between these two figures can be attributed to chance, because the dilution X antibiotic interaction was not significant. The mean square for this interaction was less than that for error. Therefore, it can be concluded that the quantitative differences obtained previously with sulfanilamide (11) still apply when antibiotics are added. These earlier results indicated that one can expect a sacrifice of approximately 3 per cent in non-return rate when increasing the dilution level from 1 : 100 to 1 : 200. These levels correspond, on the average, to a reduction in spermatozoan numbers from approximately 12 to 6 million per milliliter, the volume customarily used per insemination. Insufficient data were available to establish quantitative figures for lower spermatozoan numbers. In the experiments reported in this paper, levels of 1 : 100 and 1 : 300 were compared in order to have greater differences and a greater likelihood of establishing significance than if 1 : 100 and 1 : 200 levels were studied. If the effect of the presence or the absence of antibiotics is the same with 1 : 100 as with 1 : 300, the same certainly should apply with 1 : 100 and 1 : 200.

In both experiments, the fertility of some bulls appeared to be benefited by antibiotics to a greater extent than others. In experiment II, which was designed to measure bull X antibiotic interaction, this interaction was not significant. None of the other interactions in the variance analyses of the two experiments combined was significant.

DILUTION EFFECT

Branton *et al.* (1) have indicated that it is desirable to extend semen on the basis of spermatozoan numbers, rather than dilution level, because of the great variability in spermatozoan concentration in ejaculated semen. Therefore, experiments comparing different levels of spermatozoan numbers probably would be of more direct application to the field than those studying different dilution levels. The experiments reported herein were, nevertheless, based upon dilution levels in order to have a variation in spermatozoan numbers within each level. Then one would be able to adjust mathematically the dilution means to the same spermatozoan numbers by use of covariance. This mathematical adjustment would be equivalent to making the semen at each dilution level contain the same number of spermatozoa. The only variable then remaining would be the dilution levels of 1 : 100 and 1 : 300, and if any significant difference remained, it could be attributed to dilution effect.

Results and discussion. In the two experiments prior to adjustment, there was a very highly significant difference between non-return rates for 1 : 100 and 1 : 300, as stated above. Nevertheless, when the dilution means for the two experiments combined had been adjusted to the same number of spermatozoa, there remained a slight but insignificant difference in non-return rate. These results indicate, therefore, that the difference in fertility of semen extended 1 : 100 and 1 : 300 is due entirely or in large part to number of spermatozoa and that dilution

effect is of little or no importance. When extending semen for use in the field, one is justified, on the basis of these results, in extending according to numbers of spermatozoa without regard to actual dilution level, at least to 1 : 300.

The data in these experiments were based upon breedings made 25 to 42 hr. after collection. The dilution effect might assume greater relative importance with longer storage times. The data reported by Cheng *et al.* (4) also indicated that the immediate effect of dilution upon motility of bovine spermatozoa in yolk-citrate would not be appreciable at 1 : 300. At higher levels it appeared to be of greater importance.

SPERMATOZOAN NUMBERS. EXPERIMENTAL DATA

In these two experiments a number of samples and breedings were obtained with spermatozoan counts as low as 1.5 million per milliliter. Since there was a similar range in experiment 4 of work previously reported (11), the data from that experiment were studied in addition to the two reported above. The data were divided into groups based upon class intervals of spermatozoan numbers.

TABLE 3

Non-returns from semen samples from 3 experiments grouped into class intervals of spermatozoan numbers per milliliter of diluted semen. Experimental data.

Millions of sperm/ml.	Samples	Services	Non-returns
	(*no.*)	(*no.*)	(%)
1.5–1.9	4	226	52.7
2.0–2.9	26	960	55.6
3.0–3.9	41	1,857	59.6
4.0–4.9	35	1,526	63.6
5.0–5.9	20	928	63.3
6.0–7.9	27	1,239	63.8
8.0–9.9	29	1,271	65.1
10.0–11.9	18	1,038	70.0
12.0–13.9	18	815	72.3
14.0–21.7	18	885	68.2
Totals & av.	236	10,745	63.8

These class intervals were varied in order to include a somewhat similar number of breedings in each. Since the trends in the three experiments were very similar, only the combined data are presented in table 3.

Results and discussion. There was an over-all downward trend with decrease in spermatozoan numbers. This trend was gradual to 4 million spermatozoa. Below this number non-return rate declined rapidly. It is surprising that a non-return rate of 53 per cent was obtained with samples containing as few as 1.5 to 1.9 million spermatozoa per milliliter.

An analysis of the data in the range of 4 million and above indicated that they were linear because the individual interval averages did not deviate significantly from the regression line. Regression analysis for this range of data indicated that there was a decline of 0.6 per cent in non-return rate with each reduction of one million spermatozoa inseminated. This regression coefficient of 0.6 was highly significant. The regression coefficient for the range of 4.9 million

spermatozoa and below was 3.9 and was highly significant. However, the over-all data were significantly curvilinear. By use of the coefficient of 0.6, the calculated decline in non-return rate when reducing spermatozoan numbers from 12 to 6 million would be 3.6.

SPERMATOZOAN NUMBERS. FIELD DATA.

After a major portion of the data in table 2 had been obtained, two artificial breeding organizations adopted the practice, on a trial basis for limited periods of time, of shipping semen containing as few as 4 million spermatozoa per milliliter. This was done to study the feasibility of such a practice at occasional instances where a bull of a given breed gave insufficient spermatozoa to enable shipment of semen with a count of at least 12 million. By reducing spermatozoan numbers the necessity of using a second bull of the same breed on the same day was avoided. A few samples from one stud actually contained only 2.4 to 2.9 million sperm per milliliter.

TABLE 4

Non-returns from semen samples grouped into class intervals of spermatozoan numbers per milliliter of diluted semen. Field data

Millions of sperm/ml.	Samples	Services	Non-returns
	(*no.*)	(*no.*)	(%)
2.4–2.9	3	732	49.0
3.0–3.9	13	2,335	57.8
4.0–4.9	29	5,365	58.6
5.0–5.9	35	5,613	62.5
6.0–7.9	102	18,846	63.6
8.0–9.9	75	12,657	65.8
10.0–11.9	68	10,593	66.0
12.0–13.9	72	9,572	66.6
14.0–31.2	54	6,623	67.9
Totals & av.	451	72,336	64.3

In general, the procedure followed in the processing of semen was similar to that followed in obtaining the data for table 1. Antibiotics were added to the diluter at the rate of 500 units or micrograms per milliliter. The collections were not, of course, split. Breeding efficiency was measured by 60- to 90-day non-returns to first services made the day following collection.

The collections from these periods were grouped into the same class intervals for spermatozoan numbers as were the data in table 2, and the non-return rate for each interval was computed. Since the results from the two studs were very similar, only the combined data are given in table 4.

Results and discussion. The over-all average non-return rate was 64.3 per cent, while that for samples containing 12 million or more spermatozoa per milliliter, averaged 67.2 per cent. It appears, therefore, that about 3 per cent in breeding efficiency was sacrificed by following the practice of shipping semen containing as little as 4 million spermatozoa per milliliter when necessary, rather than shipping semen containing no less than 12 million.

The data in table 4 approach the form of a smooth curve. There is a gradual

decline in breeding efficiency as spermatozoan numbers are reduced to 8 million. As numbers are reduced from 8 to 4 million there is an increasing rate of decline in breeding efficiency. Below 4 million there is a very rapid decline in breeding efficiency with reduction in spermatozoan numbers. A similar rapid decline below 4 million was observed in table 3. In previous work (11) with comparatively limited data, rapid decline in fertility appeared to begin just below 6 million. The individual averages of the intervals above 4 million in table 4 deviate significantly from the regression line, indicating that the data definitely are curvilinear. All the data together are, of course, also significantly curvilinear.

When reducing numbers from 12.0–13.9 to 6.0–7.9 million, there is a sacrifice of 3 per cent in non-return rate. This figure agrees almost exactly with that obtained in a previous study (11) and agrees fairly well with the figure of 3.6 derived from the data presented in table 3.

In tables 3 and 4 the non-return rates with the different spermatozoan concentrations and the over-all trends may not be representative of results that might be expected with all bulls in the population. Some bulls were represented at the different concentrations more than others. A careful study of the data by individual bulls indicated that any errors that might be caused by this bias are small in magnitude.

As table 3 indicates, a non-return rate of 53 per cent resulted with only 1.5 to 1.9 million spermatozoa per milliliter. In the field data in table 4, with 2.4 to 2.9 million spermatozoa there was a non-return rate of 49 per cent. These data suggest that complete sterility will be approached with numbers of spermatozoa considerably less than those in these studies. It is of interest that workers cited by Chang (2) and Cheng and Casida (3) found that 100,000 spermatozoa or less were required per insemination to produce complete sterility in rabbits.

SUMMARY AND CONCLUSIONS

Two controlled, factorial experiments involving 6,407 services from 32 collections from nine bulls were conducted to determine whether or not there was a different rate of decline in breeding efficiency when dilution of semen was increased from 1:100 to 1:300 with yolk-citrate-sulfanilamide-penicillin-streptomycin than without the antibiotics. There was a slight and insignificant difference. Therefore, the decline in breeding efficiency with increase in dilution level can be assumed to be the same whether or not antibiotics are used.

Although there was a very highly significant difference in non-return rate for 1:100 and 1:300, this difference was no longer significant when the non-return averages were adjusted to the same spermatozoan numbers by means of covariance. These results indicate that spermatozoan numbers are much more important than dilution effect in influencing breeding efficiency at the dilution levels studied.

Addition of antibiotics caused a significant improvement in breeding efficiency over samples not containing them. There was evidence that some bulls were benefited more than others, but such bull differences could not be definitely established.

Non-return rates from three experiments representing 10,745 services were calculated for different numbers of spermatozoa per milliliter of diluted semen. The data for 4 million or more spermatozoa per milliliter were linear. The regression coefficient for this range was 0.6. As spermatozoan concentration was reduced below 4 million, the decline in breeding efficiency was much more rapid. The regression coefficient was 3.6. An average non-return rate of 53 per cent was obtained with samples containing 1.5 to 1.9 million spermatozoa per milliliter.

Field data from 72,336 services were studied in a similar manner. These data as a whole and also those for 4 million or more spermatozoa per milliliter were curvilinear. There was a gradual decline in breeding efficiency as spermatozoan numbers were reduced from 12 to 8 million, a more rapid decline as numbers were reduced from 8 to 4 million, and a very rapid decline as numbers were reduced below 4 million. An average non-return rate of 49 per cent was obtained with semen containing 2.4 to 2.9 million spermatozoa per milliliter.

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THE EFFECT OF SODIUM ARSENITE ON THE RESPIRATION OF BOVINE SEMEN AND THE RELATION OF THIS RESPONSE TO THE FERTILIZING ABILITY OF SEMEN[1]

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The effect of sulfhydryl reagents such as sodium arsenite on the respiration of semen is of physiological importance because the response is related to regulatory enzymes of metabolism and to enzyme systems essential to the maintenance of metabolism. Low concentrations of sodium arsenite stimulate respiration, while higher concentrations inhibit respiration in sea urchin sperm (Barron *et al.*, 1). The stimulatory effect is presumed due to the inactivation of the regulatory -SH groups by the sulfhydryl reagent. The inhibitory effect by greater concentrations is due to the inactivation of less readily accessible -SH groups in the protein of the cells. Thus the concentration of sodium arsenite required to bring about a stimulation of respiration should roughly indicate the amount of soluble -SH or regulatory -SH groups in the semen. The regulatory mechanisms should play a role in the fertilizing abilities of the semen.

The purpose of this experiment was, first, to determine the concentration of sodium arsenite that was necessary to bring about a stimulation of respiration in bovine semen and, second, to determine the relationship of this response in the individual semen sample to its fertilizing ability.

METHODS

The semen used in these experiments was obtained from the bull stud at the University of Nebraska. Oxygen uptake determinations were made by the direct method (2), using a Barcroft-Warburg apparatus. Dilution and treatment of the semen was carried out and the O_2-use determinations started as soon as practical after collection of the semen, usually within 1 hr. In the study involving fertility of the semen, a composite sample of the total ejaculates shipped to breeding associations was used in the O_2 determinations. Control and semen treated with sodium arsenite were diluted at the same rate, using a phosphate buffer made up of 2.0 g. $Na_2HPO_4 \cdot 12\ H_2O$ and 0.2 g. KH_2PO_4 per 100 ml. distilled water (pH 7.2). The phosphate buffer used to dilute the semen treated with sodium arsenite contained a concentration of the salt designated in the discussion of results. Since the spermatozoa concentration in the original semen varied with the individual sample, the dilution with arsenite-containing diluter was adjusted so that a constant concentration of sodium arsenite per unit number of sperm was maintained within a certain series. Later exploratory experiments

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showed that there was no difference in the variation of respiratory response to sodium arsenite caused by the small difference in sodium arsenite concentration as a result of variation of the sperm concentration between semen samples so that following experiments were diluted 1 : 5 regardless of sperm concentration in the original semen.

The respiratory response of bovine semen to added arsenite is expressed as the percentage that the O_2 uptake of arsenite-treated semen was of O_2 uptake of semen diluted at the same rate with no arsenite. Fertility estimates of individual semen samples were determined by the per cent non-returns to first service after a 60- to 90-day period.

Most of the determinations reported on in this paper were carried out for the period from March through August. This is mentioned because there are indications that there may be a seasonal influence on the O_2 uptake response to sodium arsenite.

RESULTS

The O_2 uptake response of individual bovine semen samples to a definite arsenite concentration is extremely variable (table 1). The mean O_2 uptake of 70 semen samples treated with arsenite in a concentration of 5×10^{-10} M was 111.8 per cent of the control, with a standard deviation of 29.8 percentage units. Actual variation in this series was from 65 to 208 per cent of control. Fifty-seven semen samples treated with a 10^{-5} M solution of sodium arsenite had a mean response of 97.1 per cent of control O_2 uptake values and a standard deviation of 17.5 percentage units. In this series actual variation was from 60 to 150 per cent of control values. Twenty-five semen samples treated with a 10^{-3} M solution sodium arsenite had a mean O_2 uptake 53.0 per cent of the control, with a range of from 10 to 80 per cent.

The effect of low and high concentrations of a buffered sodium arsenite solution was determined on the same semen samples. A 5×10^{-10} M solution resulted in a mean increase of 12.3 per cent in the O_2 uptake while a 10^{-5} M solution decreased the mean O_2 uptake to 96.2 per cent of control (series D, table 1). In another series, the effect of a 5×10^{-10} M and a 10^{-3} M solution of arsenite was determined on the same semen samples (series E, table 1). The 5×10^{-10} M solution increased the mean O_2 uptake of this group of semen samples 8.6 per cent above control while the 10^{-3} M solution decreased mean O_2 uptake to 53 per cent of the control. Here again wide variation in response between individual samples occurred.

These experiments indicate that the respiration rate of bovine semen is increased in many instances by low concentrations of sodium arsenite and decreased by high concentrations. The response to a definite concentration as used in these experiments is dependent to a large extent on the individuality of the semen sample.

Since it was thought that the difference in response to arsenite by individual semen samples probably is dependent upon some basic difference in the respiratory mechanisms between semen samples, the relationship of the response per-

centage to fertility was studied. The relationship between respiration response of 70 semen samples to a 5×10^{-10} M solution of sodium arsenite and fertility estimates on an individual semen sample basis is shown by a correlation coefficient of -0.410. Statistically this relationship is highly significant. The regression coefficient of fertility on response to sodium arsenite in this series is

TABLE 1

Effect of sodium arsenite on respiration of bovine semen

SERIES A	O_2 uptake				
No. semen samples	Untreated control (mm.³/hr. per 10^8 sperm)	Arsenite 5×10^{-10} M (mm.³/hr. per 10^8 sperm)	Mean $\frac{O_2 \text{ arsenite}}{O_2 \text{ control}}$	Standard deviation percentage units	Range of response $\frac{O_2 \text{ arsenite}}{O_2 \text{ control}}$
			(%)		
70	8.10	9.56	118.0	29.8	65 to 208
SERIES B	O_2 uptake				
No. semen samples	Untreated control (mm.³/hr. per 10^8 sperm)	Arsenite 10^{-5} M (mm.³/hr. per 10^8 sperm)	Mean $\frac{O_2 \text{ arsenite}}{O_2 \text{ control}}$	Standard deviation percentage units	Range of response $\frac{O_2 \text{ arsenite}}{O_2 \text{ control}}$
			(%)		
57	7.81	7.58	97.1	17.5	60 to 150
SERIES C	O_2 uptake				
No. semen samples	Untreated control (mm.³/hr. per 10^8 sperm)	Arsenite 10^{-3} M (mm.³/hr. per 10^8 sperm)	Mean $\frac{O_2 \text{ arsenite}}{O_2 \text{ control}}$	Standard deviation percentage units	Range of response $\frac{O_2 \text{ arsenite}}{O_2 \text{ control}}$
			(%)		
25	8.32	4.28	51.4	18.0	10 to 80
SERIES D	Comparison of 5×10^{-10} and arsenite and 10^{-5} M arsenite on same semen				
No. semen samples	Untreated control (mm.³/hr. per 10^8 sperm)	Arsenite 5×10^{-10} M (mm.³/hr. per 10^8 sperm)	Arsenite 10^{-5} M (mm.³/hr. per 10^8 sperm)	5×10^{-10} M % of control	10^{-5} M % of control
40	8.62	9.68	8.29	112.3	96.2
SERIES E	Comparison of 5×10^{-10} M and 10^{-3} M arsenite on same semen				
No. semen samples	Untreated control (mm.³/hr. per 10^8 sperm)	Arsenite 5×10^{-10} M (mm.³/hr. per 10^8 sperm)	Arsenite 10^{-3} M (mm.³/hr. per 10^8 sperm)	5×10^{-10} M % of control	10^{-3} M % of control
25	9.49	10.31	5.03	108.6	53.0

-0.14. This regression is significant statistically but too low to be of practical value for prediction purposes. In the series of determinations using a 10^{-5} M solution of sodium arsenite (57 samples), the relationship between percentage O_2 uptake response and fertilizing capacity is expressed by a correlation coefficient of -0.323. This coefficient also is significant.

To obtain additional evidence on whether or not low fertilizing capacity is associated with increased response to sodium arsenite, the effect of a 5×10^{-10} M solution of sodium arsenite on O_2 uptake of 12 semen samples judged to be of too poor quality for use in artificial breeding were used for respiration response study. The mean O_2 uptake response of these samples was 156 per cent of control. They varied from 116 to 271 per cent. The results indicate that the greater the response induced by low concentrations of sodium arsenite, the less is the fertilizing capacity of the semen. There is indication that the relationship between response to sodium arsenite and fertilizing capacity of the semen is curvilinear rather than linear. If it is curvilinear, it would indicate that high fertilizing capacity is partially dependent on an optimal level of regulatory -SH groups.

Since the correlation coefficient is low (-0.410 and -0.323) the fertility of semen undoubtedly is associated with other physiological as well as environmental factors. Apparently criteria in addition to the character of response to low concentrations of sodium arsenite must be taken into account to accurately evaluate the fertilizing capacity of bovine semen.

SUMMARY

Treatment of bovine semen with a low concentration of buffered sodium arsenite (5×10^{-10} M) increased the mean O_2 uptake of the semen studied. A high concentration (10^{-5} M) slightly decreased the mean O_2 uptake and a still higher concentration (10^{-3} M) decreased O_2 uptake when compared to O_2 uptake of untreated semen. The response of O_2 uptake to sodium arsenite varied greatly according to the individual semen sample.

When the individual semen sample response was related to the fertilizing capacity of the semen, a negative relationship was found. The degree of relationship was statistically significant but not close enough to be of practical value for accurate prediction of semen quality.

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THE VALUE OF AUREOMYCIN AND VITAMIN B_{12} IN MILK REPLACEMENT FORMULAS FOR DAIRY CALVES[1, 2]

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A number of reports (1, 2, 4, 5, 6, 7, 8) indicate that use of aureomycin in the rations of young dairy calves stimulates growth, decreases the incidence and severity of scours and may increase the efficiency of feed utilization. A preliminary report by Bloom and Knodt (3), relative to the use of aureomycin in milk replacements, also has indicated the value of this material in milk replacement rations of young calves.

The milk replacement formula (1) developed at this station served as a convenient medium by which different levels and types of aureomycin supplementation were studied in relation to their effects on the growth and well being of young dairy calves.

EXPERIMENTAL PROCEDURES

The male Holstein calves used in these trials were obtained from Pennsylvania state institutional herds during the late spring and summer of 1951. The calves ranged from 85 to 115 lb. in weight. Housing was in a steam-heated, ventilated and artificially lighted stable. The temperature was maintained at 65° F. by thermostatic control. All calves, except the final eight placed on trial, were assigned at random to solid-wall pens bedded with straw and equipped with a water bowl, grain mixture concentrate box, hay rack and iodized salt block. The last eight calves were selected on the basis of body weight in order to equalize the groups. In determining growth rate, measurements of body weight, withers height and chest circumference were taken once each week by the same person, starting at the same time of day and in the same order. The general appearance, vigor and feces condition of each calf were noted daily. In cases of scours which persisted longer than 24 hr., 8 g. of sulfathaladine in bolus form were given, followed by a 4-g. dose at each of the next two successive feedings.

The 48 calves in this trial were divided into eight groups of six calves each, and were placed on trial no later than 4 days after birth. The basic milk replacement formula used for all calves had the following percentage composition: Dried skimmilk, 50; distillers dried corn solubles, 15; oat flour, 5; blood flour, 10; dextrose, 7; dried whey, 10; vitamin A and D meal, 0.5; and mineral mix, 2.5. The supplements added to the basal milk replacement formula were crystalline

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vitamin B_{12}, crystalline aureomycin-hydrochloride and an aureomycin supplement which contained 2 g. of aureomycin and 2 mg. of vitamin B_{12} activity per pound. The basic replacement feed for each group was supplemented as shown in table 1.

Each replacement was dissolved in water and fed twice daily at 100° F. according to the following schedule: First through 4th day—dam's milk; 5th through 7th day—2.0 lb. whole milk, 0.2 lb. milk replacement, 2.0 lb. water; 8th through 10th day—1.0 lb. whole milk, 0.4 lb. milk replacement, 3.0 lb. water; 11th through 21st day—0.5 lb. milk replacement, 5.0 lb. water; 22nd through 35th day—0.6 lb. milk replacement, 6.0 lb. water; 36th through 49th day—0.7 lb. milk replacement, 6.0 lb. water; 50th through 56th day—0.7 lb. milk replacement, 7.0 lb. water (once daily). A fair quality mixed timothy-clover hay was fed *ad libitum* to each calf during the 12-wk. trials. Calf starter was fed *ad libitum* up to a maximum consumption of 6 lb. daily. The calf starter was composed of 416 lb. ground yellow corn, 300 lb. wheat bran, 400 lb. crimped whole oats, 100 lb. linseed oil meal, 300 lb. soybean oil meal (solvent process), 150 lb. dehydrated alfalfa meal, 100 lb. feeding cane molasses, 100 lb. dried skimmilk, 100 lb. distillers dried corn solubles, 10 lb. dicalcium phosphate, 10 lb. pulverized lime-stone, 10 lb. iodized salt and 4 lb. vitamin A and D meal (1,814,544 U.S.P. units of vitamin A and 226,800 U.S.P. units vitamin D_2 per lb.).

TABLE 1

Supplementation of milk replacements and summary of calf starter and hay consumption

Group	Supplementation/ 100 lb.	Mean daily starter		Average starter consumption			lb. starter/ lb. grain		Av. hay consumption
		Aureomycin	Vitamin B_{12}	0–4 wk.	0–8 wk.	0–12 wk.	0–8 wk.	0–12 wk.	
		(*mg.*)	(γ)		(*lb.*)				(*lb.*)
I	Control (none)			18.5	85.5	234.3	1.41	2.16	30.5
II	1 mg. crystalline vit. B_{12}		10	15.4	76.5	225.0	1.27	2.06	41.2
III	0.5 g. crystalline aureomycin-HCl	5.0		15.8	82.6	234.8	1.28	2.09	29.6
IV	1.0 g. crystalline aureomycin-HCl	10.0		24.6	106.6	265.1	1.35	2.03	19.6
V	2.0 g. crystalline aureomycin-HCl	20.0		25.3	99.4	253.9	1.32	2.06	36.7
VI	0.25 lb. Aurofac[a]	5.0	5	15.2	85.6	237.4	1.31	2.06	33.4
VII	0.50 lb. Aurofac[a]	10.0	10	20.5	95.2	247.8	1.38	1.93	33.2
VIII	1.00 lb. Aurofac[a]	20.0	20	25.2	107.3	268.6	1.35	2.04	34.7

[a] Contained 2 g. aureomycin and 2 mg. vitamin B_{12}/lb.

RESULTS

The growth data are summarized in table 2. The calves of group II, receiving the crystalline vitamin B_{12} supplement, paralleled the rate of growth made by the control replacement group and provided, in effect, a second control group. In these trials the growth stimulating effects of the highest levels of the crystalline aureomycin-hydrochloride and of the aureomycin supplement were most

pronounced at 4 wk. of age. The control and vitamin B_{12}-supplemented groups at 4 wk. had an average daily gain of 0.54 and 0.58 lb., respectively, while the groups which received the crystalline aureomycin and the aureomycin supplement had an average daily gain of 0.87 and 1.00 lb., respectively.

The analysis of variance of these data revealed that the differences in growth rates between groups were significant at the 1.0 per cent level through 8 wk. of age. The large differences in growth rates observed at 4 and 8 wk. between the control groups and the aureomycin-supplemented groups were not maintained for the duration of the experiment. At 12 wk., 4 wk. after the termination of the supplemented feeding period, the average daily gains of the control and vitamin B_{12}-supplemented groups were 1.29 and 1.30 lb., respectively. The average daily gains of group IV, that received the medial level of the crystalline

TABLE 2

Mean daily gains in body weight, withers height and chest circumference

Group	Supplementation/ 100 lb.	Body wt.			Withers ht.			Chest circumference		
		4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.
			(*lb.*)			(*cm.*)			(*in.*)	
I	Control (none)	0.54	1.09	1.29	0.12	0.14	0.14	0.06	0.09	0.10
II	1 mg. crystalline vit. B_{12}	0.58	1.08	1.30	0.10	0.13	0.14	0.05	0.08	0.10
III	0.5 g. crystalline aureomycin-HCl	0.62	1.15	1.34	0.11	0.16	0.15	0.05	0.10	0.10
IV	1.0 g. crystalline aureomycin-HCl	0.83	1.41	1.56	0.13	0.16	0.16	0.08	0.11	0.11
V	2.0 g. crystalline aureomycin-HCl	0.59	1.17	1.37	0.13	0.14	0.16	0.07	0.10	0.11
VI	0.25 lb. Aurofac[a]	0.87	1.35	1.47	0.13	0.16	0.16	0.08	0.11	0.11
VII	0.50 lb. Aurofac[a]	0.67	1.23	1.53	0.14	0.15	0.16	0.05	0.10	0.10
VIII	1.00 lb. Aurofac[a]	1.00	1.42	1.57	0.14	0.18	0.17	0.09	0.12	0.12

[a] Contained 2 g. aureomycin and 2 mg. vitamin B_{12}/lb.

aureomycin supplementation and of group VIII, that received the highest level of the aureomycin supplement, were 1.56 and 1.57 lb., respectively. The analysis of variance of these data at 12 wk. revealed that these differences in the rates of gain in weight were significant statistically ($P > 5.0$ per cent). The growth stimulatory effect apparently is due to the presence of the antibiotic and is not due to the presence of the supplementary vitamin B_{12}.

The lowest levels of both types of aureomycin supplementation produced only slight increases in growth rates over that of the controls. Group IV, which received 1 g. of the crystalline aureomycin per 100 lb. of milk replacement appeared to show a greater growth response than did group V, that received the highest level of the crystalline aureomycin. In the determination of the least significant mean differences for these data at 12 wk., it was shown that groups IV, VII and VIII exceeded the fiducial limits ($P > 5.0$ per cent). Group V barely fell below the upper fiducial limit. At this 12-wk. period, only groups IV and VIII exceeded the fiducial limits at the 1.0 per cent level, indicating the value of aureomycin supplementation of milk replacements at the levels studied in these groups.

The aureomycin-supplemented groups which made the greatest gains consumed the largest quantities of starter. The effect of aureomycin upon appetite appears to be shown best by the amounts of starter consumed to 4 wk. of age. The groups IV, V, VII and VIII at the higher levels of aureomycin supplementation consumed more starter than did the control and vitamin B_{12}-supplemented groups, for this 4-wk. period. There were no large differences, however, in the efficiency of grain utilization as shown in table 1.

Only very limited cases of scours developed among any of the groups and incidence was not related to feeding. During the first 6 wk. of life, the calves in these trials produced a more fluid feces than was thought to be desirable. This looseness appeared to differ from typical scours, in that the condition was not accompanied by other symptoms such as characteristic odor of feces, listlessness, loss of appetite, weakness and dehydration. However, with aureomycin supplementation the feces became firmer at an earlier time during the milk replacement feeding period.

One calf in group V died in the 12th week, 6 days before the end of the experimental period. Death was due to congenital hypertrophy of the heart muscle, rather than to any factor attributable to the experimental treatment received by the calf. This loss necessitated introduction of estimated values, wherever required, in the statistical analysis of the data.

SUMMARY

Addition of different levels and types of aureomycin supplementation to a milk replacement formula significantly increased the rate of gain in weight of dairy calves until they were 12 wk. of age. Aureomycin supplementation in this trial had its greatest growth promoting effect during the first 4 wk. of the replacement feeding. Aureomycin-fed calves generally were stimulated to a greater consumption of calf starter compared to their controls during the aureomycin feeding period. Aureomycin supplementation of a milk replacement feed resulted in an earlier and increased firmness of the feces. Supplementation of the milk replacement feed with vitamin B_{12} did not increase growth rates.

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A PLATING METHOD FOR THE ISOLATION AND ENUMERATION OF PROPIONIBACTERIA

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The role of propionibacteria in the development of flavor and eyes in Swiss cheese has been investigated by many workers. Ordinary plating procedures have not been of value in determining numbers of propionibacteria since the organisms will not grow under ordinary plating conditions.

von Freudenreich and Orla-Jensen (3), who first isolated these organisms from Swiss cheese in 1907, employed gelatin plates and shake cultures. Sherman (4), added dilutions of cheese to a peptone, yeast extract and lactic acid agar and then sealed this seeded nutrient medium in glass tubes.

Until 1941, the shake-culture method of growing and counting these organisms was used almost exclusively. At this time, Demeter and Janoschek (2) developed a method adapted to the use of standard petri dishes. In this method, the oxidation-reduction potential of a special yeast lactate agar was lowered with sodium sulfite. Then, overlayers of the medium and sterile paraffin oil were added. This method eliminated some of the difficulties associated with deep culture shake tubes but, obviously, is not adapted to large scale usage on a routine basis.

The following work was undertaken to develop a simple and a rapid method for the cultivation of propionibacteria.

EXPERIMENTAL METHODS

The test organism was a culture of *Propionibacterium shermanii* obtained from the Bureau of Dairy Industry. This organism, which was used throughout the experiment, is routinely used in the manufacture of Swiss cheese. Broth cultures were prepared by adding a 1.0 per cent inoculum to a broth containing 5 g. Difco yeast extract, 5 g. glucose, 20 g. BBL Trypticase and 1,000 ml. of distilled water. Before inoculation, the broth was adjusted to pH 7.0, dispensed in 100-ml. amounts in screw-cap dilution bottles and autoclaved at 121° C. for 20 min. Cultures were incubated at 30° C. for 48 hr.

A nutrient medium of the same composition as the broth plus 1.5 per cent agar was employed for plating. Concentrations of sodium thioglycollate in this medium were varied according to the experimental design. This agar, hereafter referred to as the "bottom layer" was dispensed in screw-cap test tubes in 9-ml. amounts and autoclaved at 121° C. for 20 min.

"Top layers" were prepared by the addition of varying amounts of sodium thioglycollate and agar to distilled water. These mixtures were dispensed in 70-ml. amounts in screw-cap dilution bottles and were autoclaved for 30 min. at 121° C.

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All glassware used for plating conformed to standard A. P. H. A. specifications as set forth in *Standard Methods for the Examination of Dairy Products* (1). All plates were incubated for 10 days at 30° C. Colony counts were made with the use of a dark-field Quebec colony counter.

To determine which concentrations of sodium thioglycollate and agar in the top and bottom layers would give the highest total count, broth cultures were plated according to the following procedure. Appropriate dilutions of a broth culture were introduced into sterile petri plates. Nine-ml. portions of the bottom layer medium (melted and cooled to 45° C.) were added to these dilutions and the plates rotated to insure uniform colony distribution. When the bottom layers solidified, 70-ml. portions of the melted top layer were poured into each petri dish. The top layers were allowed to solidify and the petri dishes were then inverted and incubated for 10 days at 30° C.

The first condition to be studied was the effect of varying the concentration of agar and sodium thioglycollate in the top layer. By using concentrations of 0.0, 0.05, 0.1, 0.2, 0.3 and 0.5 per cent sodium thioglycollate

TABLE 1

Effect of variation of agar and sodium thioglycollate concentration in top layer

Combination	% Sodium thioglycollate	% Agar	Total count[a]
13	0.0	1.5	Colonies too small to count
14	0.05	1.5	55.4
15	0.1	1.5	49.0
16	0.2	1.5	29.6
19	0.0	2.0	57.8
20	0.05	2.0	55.1
21	0.1	2.0	30.6
22	0.2	2.0	39.0
25	0.0	2.5	Colonies too small to count
26	0.05	2.5	55.7
27	0.1	2.5	46.8
28	0.2	2.5	39.9

[a] Counts represent average of 10 replicates, expressed as multiples of 100 million.

RESULTS

in six different concentrations of agar (0.75, 1.0, 1.5, 2.0, 2.5 and 3.0 per cent), 36 different top layers were studied. Two general trends seemed to exist. An increase in agar concentration in the top layer was followed by general increases in total counts. Increases in the concentration of sodium thioglycollate in the top layer resulted in lower total counts of the test organism. Agar concentrations of less than 1.5 per cent produced top layers that were too soft to handle; concentrations of agar over 2.0 per cent produced a cloudy discoloration in the top layers that partially obscured the colonies. This experiment was repeated employing only 1.5, 2.0 and 2.5 per cent agar in the top layer. Concentrations of 0.0, 0.05, 0.1 and 0.2 per cent sodium thioglycollate again were used. Data are presented in table 1.

A concentration of 1.5 per cent agar in the top layer again afforded better

colony visibility. As was noticed in the first survey experiment, those combinations without benefit of sodium thioglycollate produced colonies considerably smaller than the other series. In every instance, 0.05 per cent sodium thiocollate produced more uniform colony counts and sizes. A combination of 1.5 per cent agar and 0.05 per cent sodium thioglycollate in the top layer appeared to be the most satisfactory.

TABLE 2

Effect of variation of sodium thioglycollate concentration in top and bottom layers

% Sodium thioglycollate		Total count	
Top layer	Bottom layer	Trial A[a]	Trial B[a]
0.0	0.0	98.8	93.0
0.0	0.025	106.2	96.4
0.05	0.025	85.5	72.3
0.05	0.0	82.0	68.8

[a] Counts represent average of 10 replicate plates, expressed as multiples of 100 million.

After having selected the most desirable concentration of sodium thioglycollate in the top layer, the use of sodium thioglycollate in both layers and in the bottom layer exclusively was then considered. Results are presented in table 2. In this experiment, growth was obtained in the series in which sodium thioglycollate was not added to either the top or bottom layer. The colonies, however, were exceedingly small in size and could be counted only with difficulty. All other combinations produced colonies of average to large diameter. Of these combinations, the trials employing 0.025 per cent sodium thioglycollate in the bottom layer only gave the highest total counts.

TABLE 3

Variation of sodium thioglycollate concentration in bottom layer with plain top layer

% Sodium thioglycollate in bottom layer	Total count[a]
0.0	128.2
0.025	133.8
0.05	139.8
0.1	136.0
0.2	133.5
0.3	137.3
0.4	141.6
0.5	133.1
0.75	134.6
1.0	84.0

[a] Counts represent average of 10 replicate plates, expressed as multiples of 100 million.

Table 3 presents the differences in total counts obtained through variation of sodium thioglycollate concentration in the bottom layer. In this experiment, the top layer consisted of 70 ml. of plain 1.5 per cent agar per plate. Only the highest concentration of sodium thioglycollate (1.0 per cent) showed any appreciable reduction in total count. Differences in colony sizes were apparent.

Bottom layers containing 0.0, 0.025 and 0.05 per cent sodium thioglycollate yielded colonies comparatively small in size. Levels of 0.1, 0.2 and 0.3 per cent produced colonies about 0.25 mm. in diameter. The remaining levels, 0.4, 0.5, 0.75 and 1.0 per cent permitted development of colonies, 0.5 to 0.75 mm. in diameter.

To evaluate properly the results of this and the preceding experiments, another plating trial was conducted. Results are presented in table 4. Where the reducing agent was not used, the resulting colony size was substantially reduced. Employing 0.05 per cent sodium thioglycollate in the top layer produced numerous large-sized colonies. A bottom layer concentration of 0.5 per cent sodium thioglycollate gave uniformly large colonies. Increasing the concentration to 1.0 per cent reduced the colony count but had no influence on colony size. The 2.0 per cent level proved to be strongly inhibitory in relation to both colony size and number.

TABLE 4

Variation of sodium thioglycollate concentration in bottom layer

% Sodium thioglycollate		Total count[a]
Bottom layer	Top layer	
0.0	0.0	54.2
0.5	0.0	83.1
1.0	0.0	45.1
2.0	0.0	7.6
0.0	0.05	88.0

[a] Counts represent average of 20 replicate plates, expressed as multiples of 100 million.

As evidenced by the total counts obtained, either 0.05 per cent sodium thioglycollate in the top layer or 0.5 per cent in the bottom layer could be used with equal results. However, when the reducing agent is employed in the bottom layer, greater uniformity in colony size will result. For this reason, the use of a plain 1.5 per cent agar top layer and a bottom layer containing 0.5 per cent sodium thioglycollate is recommended.

Unstratified controls were prepared during survey experiments. As growth was negligible, this information has not been included.

Since only one strain of *P. shermanii* was employed throughout the course of experimentation, it was necessary to determine the general adaptability of the procedure to other strains and to other species of the genus. In all, 24 different cultures were plated employing the plain 1.5 per cent agar top layer with a 0.5 per cent addition of sodium thioglycollate to the bottom layer. These cultures included eight strains of *P. shermanii*, three strains of *P. rubrum*, three strains of *P. freudenreichii*, one strain of *P. arabinosum*, one strain of *P. petersonii*, three strains of *P. jensenii*, one strain of *P. zeae*, three strains of *P. thoenii* and one strain of *P. pentosaceum*. In every instance high counts and large-sized colonies resulted, demonstrating clearly the general usefulness of the method.

It should be emphasized that this method of growing propionibacteria is well adapted to use on a routine basis. Colonies from the plates are readily picked.

It is necessary to remove the joined top and bottom layers from the petri dishes before picking. This is done easily by running a sterile spatula between the agar and the inside edges of the petri dish and rotating the dish. After traversing the inside of the dish with the spatula, the contents are transferred to a second sterile petri dish in an inverted position. The colonies then are accessible for picking.

DISCUSSION

The propionibacteria have a strong tendency toward anaerobiosis. Once growth has been initiated this tendency seems to lessen. For this reason, it is necessary to employ a reducing agent in the plating medium to promote proper conditions for the initial growth phase. The application of this principle involves several interrelated factors. The reducing agent must be non-toxic at the concentrations employed. If incorporated in the plating medium it must be water-soluble, relatively stable under the conditions of use and should not obscure colony development. In addition, it should produce the desired oxidation-reduction potential for a sufficient period of time to permit and sustain colony development. Sodium thioglycollate proved to be acceptable for the purposes of this experiment. Numerically equal colony counts were obtained by using either 0.05 per cent sodium thioglycollate in the top layer or 0.5 per cent in the bottom layer. This is not surprising when it is considered that the first method uses 0.035 g. of reducing agent per plate compared with 0.045 g. for the second method. This difference is not great. In either instance, the sodium thioglycollate should diffuse evenly throughout the agar mass. However, greater uniformity in colony size was obtained when the reducing agent was applied in immediate contact with the bacterial cells.

SUMMARY

A simple, convenient and comparatively rapid method for the cultivation of members of the genus *Propionibacterium* has been developed. The method is based upon the reduction of the oxidation-reduction potential of a nutritive plating medium. This reduced potential is protected by stratifying with a layer of plain agar.

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EFFECTS OF THE HYDROTRON ON MILK AND MILKSTONE FORMATION[1]

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Formation of milkstone on milk heating equipment is the cause of one of the most important problems with which the milk plant operator must cope, namely, the cleaning of heating equipment. Milkstone is a complex heterogeneous mixture of organic and inorganic substances that adhere tenaciously to milk heating surfaces. Use of high temperature-short time (HTST) pasteurizing equipment has increased greatly the milkstone problem, because in these units large volumes of milk are heated to high temperatures in relatively confined spaces.

Milkstone should be removed from milk heating surfaces for several reasons: (a) It acts as an insulator when deposited in several layers, thus greatly reducing heat transfer, (b) it is the most common cause of high thermoduric counts in pasteurized milk, (c) it can serve as a source of off-flavors and odors, (d) it is unsightly, (e) when allowed to accumulate, it is difficult to remove.

Milkstone is similar to boiler scale. Both are formed as a result of heat treatment and are composed primarily of minerals precipitated from solution by heat.

An English engineer, A. G. Freeborn (3), has developed and patented an instrument known as the Aquastat in England, which prevents boiler scale formation in all types of steam generators. In the United States this instrument is sold under the name of Hydrotron. The instrument uses a small electrical current to charge a conducting cell through which the water passes, and this treatment affects the crystalline structure of the minerals when they are precipitated. Instead of a hard adhesive scale formed by heating, a thin friable deposit is formed which is removed easily in the ordinary blowdown. Freeborn and Willey (6) proposed a theory which explains the observed effects of treating water with small electrical currents. Their theory is as follows: "When a salt is in solution, it may take the form of ions, or molecules or both, but let us call whichever of these forms it is in, radicles. It is a well known fact that these radicles are hydrolised, or have a film of water round them, which film has a certain electrical resistance. If this hydrolised radicle comes into contact with an electrode which is charged to just a sufficiently high potential to break down the resistance of this film and no more, such resistance will be broken down and a static charge of electricity will be imparted to the radicle; but as the polarity of the charge and the electrode are similar, it follows that the electrode will commence repelling the radicle as soon as it acquires a fraction of its ultimate charge, and will completely repell it before it can acquire the full potential of the electrode. It regains its film of water and cannot discharge itself because its self-contained charge

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is not sufficiently high to break down the tension of its enveloping film. We then have all the radicles in the water with a static charge of electricity of the same polarity, entrapped by an enveloping film of water, so that the solution will be of a nature which is somewhat similar to a hydrophilic sol."

The object of this study was to investigate the possibilities of preventing milkstone in milk heating equipment by means of the Hydrotron and to observe the effects of Hydrotron treatment on the properties of milk.

EXPERIMENTAL METHODS AND EQUIPMENT

This study was divided into two phases. The first involved the use of equipment representing low-temperature, long-holding pasteurization, and the second the HTST method. Two general procedures were used.

Low-temperature, long-holding. All milk used in these experiments was good quality, fresh raw whole milk obtained from the New Jersey Agricultural Experiment Station milk plant. The cold milk was poured into a 50-gal. pasteurizer and completely mixed. While still being agitated, the milk was pumped vertically through a Hydrotron unit and collected in pails. The desired samples then were taken and immediately heat treated in a multiple-unit laboratory pasteurizer. This consisted of ten 1,000-ml. stainless steel beakers immersed in an electrically heated and controlled water bath, agitated by propellers driven from a common belt and pulley arrangement. Cooling was done by circulating ice water around the beakers.

High-temperature, short-time. The raw milk was placed in the 50-gal. holding tank and mechanically agitated. In these trials the vat was used to preheat the sample of milk, if this was desired. The milk then was pumped through the Hydrotron by means of a Waukesha variable-capacity positive pump. From the Hydrotron, the milk was passed through a helical-groove heat exchanger designed by the Bureau of Dairy Industry of the United States Department of Agriculture (4). Finally, the milk was cooled by passing it over a brine surface cooler.

The Hydrotron. The principal parts of the unit are machined from composition "M" Naval bronze castings. This material is used to provide ample strength and corrosion resistance with superior electrical characteristics. For use with milk these units were tin or zinc plated. There are no moving parts and the only expendable part of the unit is the battery. Figure 1 shows the inner tube *a*, which contains a number of vertical slots for subdividing the milk into thin streams. These slots also decrease the resistance to flow so that the pressure loss through the unit is less than 0.2 lb. per in.^{2}. The inner tube is connected to the positive terminal *b* of the electrical circuit, which is operated by a 1.5-volt dry cell housed in a control box. The body of the unit *c* is connected to the negative terminal *d* of the circuit. The electrical circuit is completed when milk flows through the apparatus. The electrical current through the Hydrotron is limited to a few milliamperes and resistors which are included in the circuit bringing the operating potential down to only a few millivolts.

The heat exchanger. The helical-groove heat exchanger (4) contains 13.6 in.^{2} of heating surface and has a capacity of 6.69 in.^{3}. The length of the helical

groove is 12 ft. When milk is pumped through the exchanger at the rate of 1,000 lb. per hour, it passes through the heating groove in 0.87 sec. with a velocity of 13.8 ft. per second. Figure 2 shows how the milk is heated with steam contained in a jacket surrounding the inner core. It also is heated by steam from inside the hollow core. Leakage of milk from one groove to another is prevented by a machined fit between the outer jacket and the coil insert.

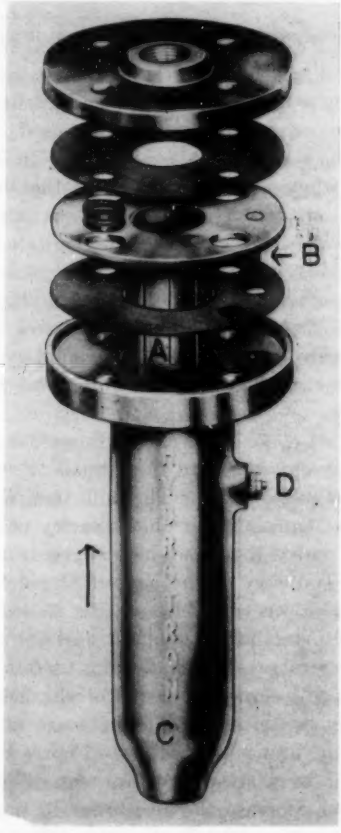

FIG. 1. An exploded view of the Hydrotron. a = inner tube; b = positive pole of circuit; c = housing of unit; d = negative pole of circuit.

EXPERIMENTAL RESULTS

Effect of the Hydrotron on milkstone. When milk was heated in stainless steel beakers to temperatures of 145, 155, 165 or 175° F. with no holding, there was very little visible difference between the film formed on the beaker by the Hydrotron-treated and control samples. The heat application in these trials was very slow, the heating water and the milk being cold at the beginning of the

trial. Under the same heating conditions as above, Hydrotron-treated milk held at 170° F. for 30 to 60 min. showed some differences in milkstone formation. Generally, the treated sample had approximately one-fourth as much precipitate as the control.

At this point in the study, trials were made to determine the milliampere setting of the Hydrotron controller which would retard milkstone deposition to the greatest extent. A current of 1 to 2 milliamperes produced the maximum difference between treated and control samples. Settings below and above this range resulted in amounts of milkstone greater than or equal to the control.

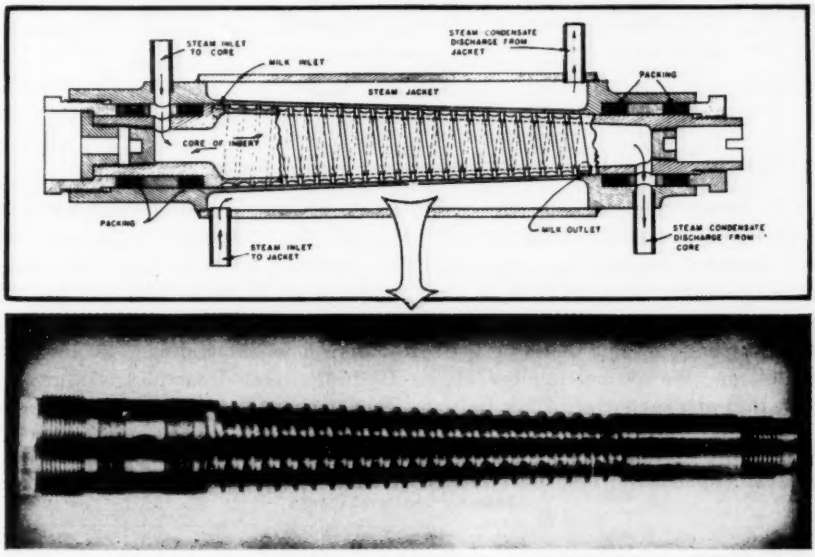

FIG. 2. Assembly and coil insert of Bureau of Dairy Industry heater. Top: Section drawing of heater. Bottom: Helical-coil insert.

Several heating trials were run with whey produced by adding rennet extract to skimmilk. Some of the whey was passed through the Hydrotron before being heated to 171 or 174° F. for 30 min. The results, as shown in table 1, indicate considerably less precipitate from the treated whey.

By using the helical-groove heat exchanger, temperatures in the range of 166 to 189° F. were obtained. Although these temperatures are higher than those usually attained in short-time units they were used because it is in the higher ranges of temperature that the Hydrotron theoretically is most effective. The results of three trials (table 2) showed that the treated samples formed milkstone in amounts approximately one-third less than the control.

A multiple run was made to produce as much milkstone as possible in the heat exchanger. In this trial the milk was recirculated through the heater until the temperature was raised from 38 to 152° F. This continuous run substantiated

the results of the other trials. The Hydrotron-treated milk formed less precipitate in the heating groove.

Effect of the Hydrotron on protein stability. In preliminary consideration of the effects which the Hydrotron treatment of milk might produce, it was thought that the proteins probably would be affected in some manner, since they are among the most active chemically and physically of the substances found in milk.

TABLE 1

Effect of Hydrotron treatment on the mineral precipitation of heated whey

Trial	Heating time	Final temp.	Holding time	Milli-amperes	Mineral precipitate[a]	
					Treated	Control
	(*min.*)	(° *F.*)	(*min.*)			
1	8	171	30	0.96	+	+++
2	8	171	30	0.96	+	+++
3	14	174	30	0.96	++	+++

[a] + = slight precipitate; ++ = definite precipitate; +++ = pronounced precipitate.

To detect changes in protein stability, alcohol number determinations were made by the procedure of Corley (1). The HCl test of Storrs (5) also was used as a means of determining protein stability; however, when the milk was heated to temperatures above 170° F., a clear end-point could not be obtained and the test was abandoned.

The results of this phase of the study were most interesting. In all cases, except one, the alcohol number of the Hydroton-treated samples was greater than that of the control. This indicates that the proteins of Hydrotron-treated

TABLE 2

Effect of Hydrotron treatment on the milkstone formation of milk heated in the Bureau of Dairy Industry heat exchanger

Preheating temp.	Final temp.	Milli-amperes	Milkstone formation[b]	
			Treated	Control
(° *F.*)	(° *F.*)			
130[a]	160	0.93	++	+++
135	166	0.96	++	+++
162	186	1.94	++	+++
162	188	1.94	++	+++
162	189	1.94	++	+++

[a] Average of 3 trials at each temperature.
[b] + = slight milkstone; ++ = definite milkstone; +++ = pronounced milkstone.

milk were more stable than they were before treatment. The results of the HCl test confirmed those obtained with the alcohol test up to the point where the HCl test no longer was satisfactory. Results of the alcohol number determinations, made on samples held at several temperatures for varying periods of time, are shown in table 3.

Effect of the Hydrotron on curd tension. The results of early experimental

trials showed that not only was the protein stability affected by Hydroton treatment but the curd tension was reduced. The curd tension of the milk samples was determined by the procedure of Doan *et al.* (2). The data in table 3 indicate that the treated samples had a lower curd tension than the control. The differences observed in this study are not great enough to produce soft curd milk, but are important because of the implications they suggest. The proteins are definitely modified in some manner, whether chemical or physical, to produce the constant variations observed in the curd tension.

TABLE 3

Effect of Hydrotron treatment on the protein stability and curd tension of milk

Temp. of Heating	Holding time	Alcohol number		Curd tension	
		Treated	Control	Treated	Control
(° *F.*)	(*min.*)			(*g.*)	
		Batch heating			
145[a]	0	8.18	8.05	28.5[d]	33.0
155	0	8.20	8.10	21.0	23.0
165	0	8.40	8.15	22.5	27.5
175	0	8.50	8.30	6.0	14.5
Av.		8.32	8.15	19.5	24.5
		Batch heating			
171[b]	5	9.30	9.30	7.5[d]	11.0
171	10	9.20	9.15	5.0	14.5
170	15	9.10	8.88	14.0	23.8
170	30	8.10	7.85	11.0	12.0
170	60	8.00	7.80	2.0	2.0
Av.		8.75	8.59	7.90	12.66
160[c]	0	8.40	8.20	35.0[e]	37.0
166	0	8.35	8.20	34.5	38.0
186	0	8.20	8.10	35.0	37.75
188	0	8.45	8.30	32.5	35.5
189	0	8.50	8.30	31.0	34.5
Av.		8.38	8.22	33.6	36.55

[a] Av. of 8 trials at each temperature.
[b] Av. of 4 trials at each temperature.
[c] Av. of 3 trials at each temperature.
[d] Curd tension obtained by manual operation of American Curd-O-Meter.
[e] Curd tension obtained by standard procedure for determining curd tension.

Effect of the Hydrotron on specific conductivity and on pH. Conductance is dependent upon ions in solution, and they are the result of molecular dissociation. Thus, if conductance is increased it may be due to increased dissociation of ionizable salts. In milk the conductors are the cations in solution (calcium and magnesium) and the anions such as citrate, phosphate, and chloride. It was thought that by measuring the specific conductance of milk before and after treatment with the Hydrotron some indication of its effect on the mineral salts might be obtained. No definite pattern of results could be obtained from the experiments, however, and it was concluded that the Hydrotron produced no effect upon the specific conductance of milk.

The pH of milk is the result of equilibrium reactions involving the acidic and basic constituents which for the most part are the minerals of milk. Because the minerals play a significant part in determining the pH of milk, any effect the Hydrotron had upon the minerals might possibly be detected by a change in the pH value. The results of the pH study indicate that the Hydrotron had no detectable effect upon the pH.

Results of installation of the Hydrotron on a commercial HTST unit. After it had become evident that the Hydrotron did have a definite effect on milkstone formation, a Hydrotron unit was installed in a local dairy plant. This dairy plant had a 6,000 lb. per hour Cherry-Burrell HTST pasteurizer. The Hydrotron was placed in the raw milk line so that all milk entering the pasteurizer would be treated.

TABLE 4

Results of commercial installation of the Hydrotron on milk precipitation in a short-time pasteurizer[a]

Trial	No. of gallons treated	Milli-amperes	Cleaning time (*min.*)		After circulation
			Acid	Alkali	
1a	2,970	Control	25	25	Plates clean
1b	3,970	1.0	10	20	Plates clean
2a	2,940	Control	10 + 17	20	After 10 min. of acid, precipitate still very hard and held to plates, 17 min. more of acid circulation needed
2b	3,450	1.0	10	15	Plates clean
3a	3,910	Control	10	10	Plates of regenerative section clean; heater section had gritty material in large amounts, brushing necessary
3b	4,000	1.0	10	10	Plates clean

[a] Before circulation, milkstone precipitate to 18th hot plate of regenerator, protein-like (sticky); all heater plates were gritty with mineral precipitate.

It was impossible to determine the relative amounts of precipitate formed on the press. After observation of the results for a few days, it was decided that a satisfactory method for evaluating the results of the Hydrotron treatment would be in timing the acid and alkali circulation necessary to clean the plates. As table 4 indicates, a normal operation requires 25-min. acid and 25-min. alkali circulation. With the Hydrotron in operation, this time was reduced to 10 min. of acid and 10 min. of alkali circulation. When the circulating time of the control run was reduced (trial 3), additional acid treatment was necessary to obtain the desired results. When a control run was made with only 10-min. circulating times, scrubbing of the heater section plates was necessary to clean them thoroughly. The scrubbing of the plates was not necessary, however, when the Hydrotron was used in a comparable trial.

DISCUSSION

During the course of this investigation the Hydrotron operated more effectively to prevent milkstone when the milk was heated to temperatures in the

range of 160 to 189° F. High temperatures, however, did not seem to be the only factor involved. Rapid heat transfer during heating of the milk appeared to be a prerequisite for obtaining good results with the Hydrotron.

The differences noted in precipitate formation at high temperatures where there was rapid heat transfer undoubtedly are related to the predominance of mineral precipitation occurring under these conditions. The ideal conditions for mineral precipitation occurred during the trials in which whey was used. With a correct milliampere setting on the Hydrotron, high temperatures, and the removal of interfering casein, good results were obtained, the treated whey forming considerably less precipitate. This might indicate that, under satisfactory conditions, the minerals may be affected in such a way that they do not readily precipitate when heated.

Curd tension determinations showed that the electrical current affected the protein in some manner. Further evidence of a protein reaction is shown by the stability tests. The alcohol and HCl test indicated that the proteins of a treated sample were more stable. This study did not entail the determination of what happened to the proteins to cause the lowering of the curd tension and the increase in stability. Possibly, it could be due to an increase in the electrical charge or to hydration of the protein micelle or both.

One method for increasing the hydration would involve the adsorbed ions of the protein micelle. If these ions leave the micelle as a result of exposure to the electrical field, this might increase protein hydration, since it is believed that adsorbed ions hydrate themselves at the expense of the adsorbent, and if the ions are removed from the adsorbing micelle it has the possibility of regaining its lost hydration.

On the other hand, the charge on the protein micelle possibly was slightly increased, and therefore, caused a reduction in curd tension. Electrophoretic studies of the proteins might give further indication of the truth of this statement.

It is impossible, at this time, to apply to milk the theory which explains why Hydrotron-treated water does not form boiler scale when heated (3, 6). The nature and the complexity of milk do not allow this, since not enough is known concerning the fundamental effects of small electrical currents upon minerals and proteins. It is entirely possible that several reactions occur simultaneously, and to interpret them adequately, a greater knowledge of the physical chemistry involved is needed.

CONCLUSIONS

This study has shown that the use of small electrical currents in milk has an effect upon the amount of milkstone which is formed by high-temperature short-time heating. The important factors involved seem to be the rapidity of heating, the final temperature attained and the current application from the Hydrotron.

A reaction involving the proteins is evident in the lowering of the curd tension and in the increase in protein stability of the treated samples.

The application of small electrical currents to milk does not seem to have any effect upon the specific conductivity or pH.

Under the conditions of these experiments the optimum setting for the Hydroton in the prevention of milkstone formation was found to be in the range of 1.0 to 2.0 milliamperes.

The Hydrotron made it possible to reduce the acid and alkali circulating times in a high-temperature short-time pasteurizer without the necessity of brushing the plates.

ACKNOWLEDGMENTS

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THE EFFECT OF COPPER UPON THE ACTIVITY OF BACTERIA ASSOCIATED WITH SWISS CHEESE MANUFACTURE

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The role of copper in promoting flavor deterioration in butter and fluid milk has been widely recognized. Copper also may interfere with the normal ripening of Cheddar cheese. Thus, Barnicoat (1) observed that maturation of Cheddar cheese made from milk containing from 3 to 6 ppm. of copper was abnormal and color defects developed. Stine *et al.* (7) reported that concentrations of copper in excess of 2 ppm. in Cheddar cheese caused delayed ripening and the development of metallic and oxidized flavors. Cheese made in stainless steel equipment contained only 0.5 to 1.5 ppm. of copper, whereas that made in tinned copper equipment contained from 0.5 to 33.8 ppm. The degree of contamination was dependent upon the amount of exposed copper on the equipment.

Conversely, high-quality Swiss cheese traditionally has been made in copper kettles, and presumably contains appreciable amounts of copper. Therefore, it became pertinent to an understanding of this apparent anomaly to determine the effect of copper upon the growth and activity of microorganisms commonly associated with Swiss cheese manufacture. It is the purpose of this paper to present data which demonstrate the influence of various levels of copper on the growth and biochemical activity of pure cultures of lactic acid bacteria and propionic acid bacteria commonly used as Swiss cheese starters.

EXPERIMENTAL PROCEDURE

Streptococcus thermophilus strain R, *Lactobacillus casei* strain 301, *Lactobacillus* sp. strain 302, *Lactobacillus bulgaricus*[1] strain K, and *Propionibacterium shermanii*[2] strains S-2, S-6, and S-9 were employed in this study.

S. thermophilus and *L. casei* were grown in a medium containing 10 g. lactose, 5 g. yeast extract, 5 g. Protolysate,[3] 0.2 g. K_2HPO_4 and 0.2 g. KH_2PO_4 dissolved in 1 l. double distilled (glass) water and adjusted to pH 6.5. The propionic acid bacteria were cultivated in a similar medium in which the lactose was replaced by 10 g. sodium lactate. *L. bulgaricus* and *Lactobacillus* sp. 302 were cultured in 1.5 per cent peptonized milk dissolved in double distilled water and adjusted to pH 6.5.

The above media, prepared double strength and diluted to volume with water (twice glass distilled) with allowance for copper supplements, were dispensed in 100-ml. quantities into 160-ml. Pyrex bottles. Analysis for the copper content (8) of yeast extract and protolysate indicated that media made of these components contained less than 0.01 ppm. of copper. Separately sterilized aqueous

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[1] Obtained from Dr. G. W. Reinbold, Kraft Foods Co., Glenview, Ill.

[2] Obtained from Dr. H. B. Naylor, Dept. of Dairy Industry, Cornell Univ., Ithaca, N. Y.

[3] Dry enzymic digest of casein, Mead-Johnson and Co.

$CuSO_4$ was added aseptically in calculated amounts after sterilization of the medium. The test media were inoculated with standardized inocula prepared from actively growing cultures. The cultures of lactic acid bacteria were incubated at 37° C., and the propionic acid bacteria were grown at 30 to 34° C. Aliquots of the fermentation mixtures were withdrawn at appropriate intervals for growth measurements and chemical determinations.

Growth was estimated turbidimetrically in a Lumetron photoelectric colorimeter (Model 400-A) equipped with a no. 650 filter and is expressed as optical density × 10. Total acid production was determined on the same 10-ml. aliquot. An equal volume of distilled water was added to the aliquot and the mixture titrated with 0.05 *N* NaOH to neutrality, using bromthymol blue as the indicator. These results are expressed as milliliters of 0.05 *N* NaOH required to neutralize 10 ml. of culture.

The volatile acids produced by the propionic acid bacteria were separated from the culture by distillation. A 25-ml. aliquot of medium was acidified to pH 2 with 10 *N* H_2SO_4, diluted to a total volume of 125 ml. with CO_2-free water and distilled until 100 ml. of distillate were collected (30 min.) in a volumetric flask. The distillate was transferred to a titration flask and titrated with 0.1 *N* NaOH to a phenolphthalein end point. Although quantitative recovery of the volatile acids present was not obtained by this method, the per cent recovery of the volatile acids present was constant. This was verified by using various amounts of standardized volatile acid solutions. Recovery values for acetic and propionic acids were 61.0 and 89.0 per cent, respectively. Thus, this procedure proved satisfactory for obtaining comparative data. The ratio of acetic to propionic acid present in the fermentation medium was determined by a modified Duclaux procedure described by Johnson (5).

Carbon dioxide production by the propionic acid bacteria was measured in an all glass apparatus (2) consisting of a calibrated gasometer attached to the fermentation flask. The evolved CO_2 was collected over a saturated Na_2SO_4 solution acidified to pH 2.0. Direct readings of CO_2 volume were made by adjusting a leveling bulb to correct for atmospheric pressure. These results are expressed as milliliters of CO_2.

The copper content of experimental cheese was determined by the method of Hedrick and Tracy (4).

RESULTS

Data obtained from growth trials in which the medium was supplemented with from 0 to 16 ppm. of copper suggest that 2 and 4 ppm. of copper had only slight inhibitory effect on the growth of *S. thermophilus* (table 1). Growth may have been retarded to a slight degree by higher levels of copper; however, this effect was not pronounced. Although the basal medium did not support profuse growth of *S. thermophilus*, sufficient levels of growth were obtained to permit measurement of the effect of copper.

The growth response of the lactobacillus cultures to various levels of copper (table 1) suggests that growth of *L. casei* was stimulated slightly by the addition of 2 ppm. copper. Higher copper concentrations (4 to 16 ppm.) were without

appreciable effect. The growth of *L. bulgaricus* appeared to remain essentially unaltered by the presence of copper in concentrations as high as 20 ppm. With *Lactobacillus* sp. 302, 2, 4 and 8 ppm. copper appeared to exert a slight stimulatory effect. However, copper concentrations of 16 to 24 ppm. retarded the growth rate.

The influence of copper on the growth of *P. shermanii* is shown in table 2. Rapid growth occurred in the unsupplemented medium, and this rate was retarded by the presence of copper. This effect was approximately proportional to the concentration of copper. Maximum growth of strain S-9 was attained in 49 hr. in the basal medium and in the medium containing 2 ppm. copper. In

TABLE 1

The effect of Cu upon the growth of some lactic acid bacteria associated with Swiss cheese manufacture

Incubation time	Optical density × 10						
	ppm. Cu^{++} added						
	0	2	4	8	16	20	24
(*hr.*)							
	Streptococcus thermophilus R						
28	1.35	1.30	1.25	0.95	1.10	.....	.....
55	1.70	1.45	1.35	1.00	1.35	.....	.....
101	2.00	1.85	1.70	1.30	1.50	.....	.....
168	2.15	1.85	1.75	1.55	1.75	.....	.....
336	2.70	2.25	2.30	2.10	2.20	.....	.....
	Lactobacillus casei 301						
28	0.90	0.70	0.75	0.70	0.75	.....	.....
55	1.15	1.45	1.10	1.30	1.30	.....	.....
101	1.40	1.85	1.50	1.55	1.60	.....	.....
168	1.50	1.95	1.60	1.55	1.70	.....	.....
336	1.83	2.16	1.75	1.70	1.70	.....	.....
	Lactobacillus bulgaricus K						
43	0.65	0.90	0.90	0.85	0.70	0.78	.....
78	1.23	1.50	1.58	1.60	1.58	1.47	.....
144	1.27	1.63	1.56	1.63	1.65	1.51	.....
216	1.63	1.65	1.65	1.75	1.78	1.65	.....
	Lactobacillus sp. 302						
29	1.30	1.23	1.22	1.25	0.65	.....	0.63
57	1.75	2.35	2.47	2.40	1.40	.....	1.30
195	2.70	3.07	3.05	2.95	1.78	.....	1.35
384	3.30	3.70	3.65	3.65	2.43	.....	1.80

the presence of 4 and 8 ppm. of copper, the growth rate was diminished to the extent that maximum growth was attained only after 75 and 312 hr., respectively. In the absence of copper or with low copper concentrations, the turbidity attained a maximum value early in the incubation. However, on continued incubation, this turbidity decreased so that the turbidity of all cultures was approximately the same after 336 hr. The cause of this decrease is unknown, but a similar phenomenon has been observed among several species and has been attributed to a depletion of the energy source (6). The lytic action of bacterio-

TABLE 2

The effect of Cu upon the growth of Propionibacterium shermanii

Incubation time	Optical density × 10				
	ppm. Cu^{++} added				
	0.0	0.5	2.0	4.0	8.0
(*hr.*)					
		P. shermanii S-2			
19	1.75	1.20	1.00	0.80	0.60
46	5.90	5.05	3.90	0.95	0.70
98	5.50	5.12	4.65	3.00	1.00
192	4.60	4.70	3.85	3.60	2.70
312	3.90	3.90	3.90	4.00	2.90
		P. shermanii S-6			
33	3.55	3.10	1.15	0.75	0.75
52	5.15	3.95	1.95	1.00	0.90
123	4.45	4.35	3.80	1.60	1.15
192	4.60	4.75	4.05	2.30	1.70
312	4.60	4.70	4.70	4.80	3.80
		P. shermanii S-9			
19	1.20	0.85	0.85	0.85	0.70
49	5.80	5.70	5.40	2.20	1.10
75	4.70	4.60	4.10	4.10	2.20
172	3.20	3.20	2.70	3.60	2.50
312	2.70	2.70	2.70	3.30	3.00

phage is another possibility. The results obtained with *P. shermanii* S-2 and S-6 are in essential agreement with the S-9 strain results insofar as the effects of copper on the growth rates are concerned.

The influence of copper on acid production by *S. thermophilus* is illustrated in figure 1. The titratable acidity decreased slightly with increasing levels of added copper. This is consistent with the observed effect of copper on growth. Table 3 shows the influence of copper on acid production by the various lacto-

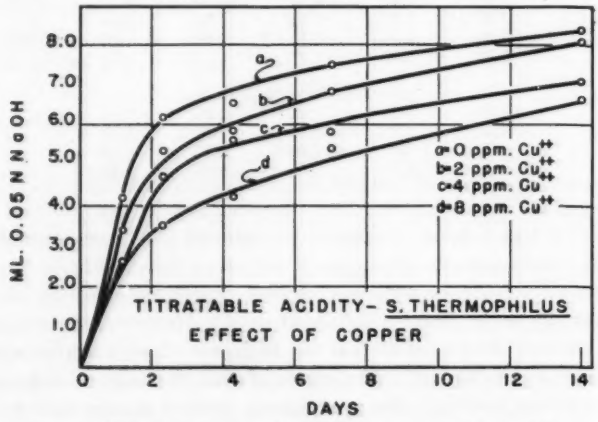

FIG. 1. Effect of Cu on acid production by *S. thermophilus*.

bacillus species. A slight increase in acid production by *L. casei* and *Lactobacillus* sp. 302 in the presence of 2 ppm. of added copper was observed. This is in agreement with the effect noted on the growth rate of these cultures. A copper concentration of 16 ppm. did not inhibit acid production by *L. casei;* however, decreased acid production by *Lactobacillus* sp. 302 was observed in copper concentrations exceeding 8 ppm. Copper levels as high as 20 ppm. did not influence the rate and extent of acid production by *L. bulgaricus.*

The rate of volatile acid production by *P. shermanii* was greatly altered by the presence of copper. The lag in volatile acid production was more pronounced with increasing levels of added copper; however, the total level of volatile acids eventually produced was approximately the same with all levels of

TABLE 3

The effect of Cu upon acid production by homofermentative lactobacilli

Incubation time	Ml. 0.05*N* NaOH/10 ml. medium						
	ppm. Cu^{++} added						
	0	2	4	8	16	20	24
(*hr.*)							
			Lactobacillus casei 301				
28	3.20	2.20	2.20	2.30	1.95	.....	.....
55	4.55	4.65	4.75	4.65	3.47	.....	.....
101	5.10	6.80	6.20	6.20	5.30	.....	.....
168	6.25	8.20	7.10	6.80	6.80	.....	.....
336	6.95	8.35	7.10	7.00	6.80	.....	.....
			Lactobacillus bulgaricus K				
43	4.47	5.00	4.56	4.47	3.83	4.00	.....
78	7.20	7.85	7.36	7.27	6.43	6.40	.....
144	8.05	7.98	7.81	7.81	7.58	7.48	.....
216	8.25	7.96	8.35	8.13	8.43	8.27	.....
556	8.30	7.80	8.35	8.40	8.40	7.70	.....
			Lactobacillus sp. 302				
29	4.32	4.40	4.25	3.95	3.55	.....	3.20
57	6.55	6.65	6.55	5.95	4.75	.....	4.20
195	8.50	9.00	8.75	8.05	5.65	.....	4.70
384	9.20	9.90	9.20	9.00	6.80	.....	5.90

copper. The observed effect varied slightly among the strains studied; however, the qualitative aspects were essentially the same in all cases. The effect of copper on volatile acid production by *P. shermanii* S-2 is illustrated by figure 2. Data from additional experiments indicated that the presence of various levels of copper in the fermentation medium did not alter the ratio of acetic to propionic acid.

The rate of CO_2 production by *P. shermanii* was retarded by the presence of copper. The lag in CO_2 production was extended with increasing levels of copper, although the total volume of CO_2 produced during the prolonged fermentation appeared to be essentially the same with all levels of copper. The influence of copper on CO_2 production was the same for the three strains of propionibacteria. Figure 3 shows the results obtained with *P. shermanii* S-2.

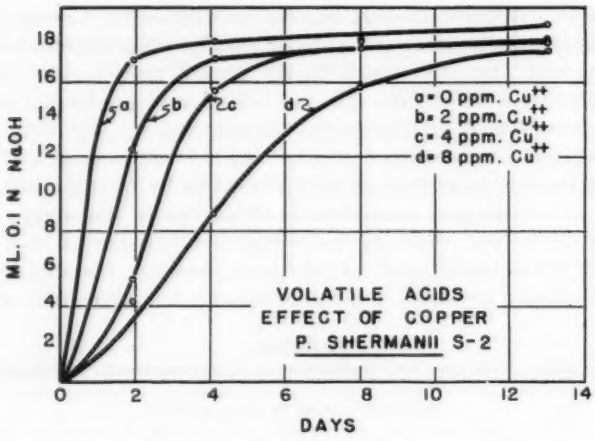

FIG. 2. Effect of Cu on volatile acid production by *P. shermanii*, S-2.

When these findings were tested in experimental Swiss-type cheese[4] (3) manufacture using *P. shermanii* S-2, *L. bulgaricus* K and *S. thermophilus* R, as starter organisms, a high level of copper (18.0 ppm.) resulted in an inferior and abnormal cheese. Copper concentrations of 7.5 to 8.0 ppm. in the cheese seemingly did not affect adversely the quality of the product. Fig. 4 shows the gross appearance of the experimental cheeses. Cheese analysis results are given in table 4.

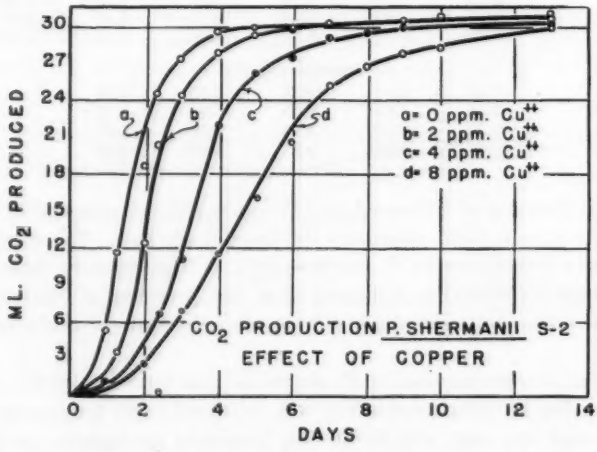

FIG. 3. Effect of Cu on CO_2 production by *P. shermanii*, S-2.

[4] Acknowledgment is made to S. L. Tuckey for manufacturing and judging the experimental cheese.

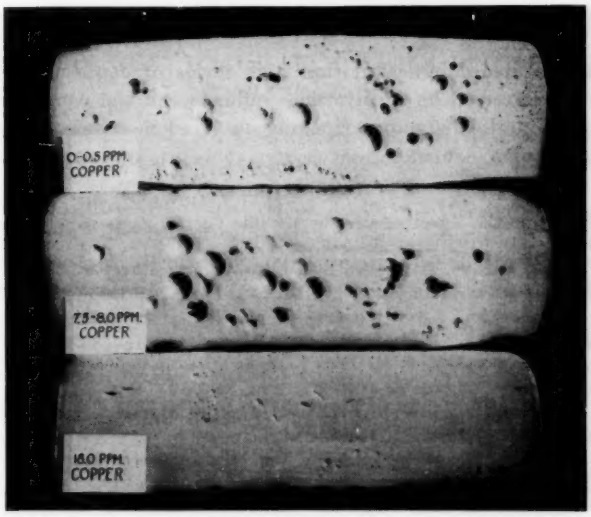

FIG. 4. Gross appearance of Swiss-type cheese after 88 days of ripening. Cu levels as indicated.

SUMMARY

Data have been presented which demonstrate that bacteria associated with Swiss cheese manufacture vary considerably in their behavior toward copper. Copper had no pronounced effect upon the growth and acid production by *S. thermophilus* and three species of homofermentative lactobacilli. Conversely, copper increased the lag phase and decreased the growth rate of the three *P. shermanii* cultures. A similar lag in volatile acid and CO_2 production was observed. These effects were roughly proportional to the copper concentration in the growth medium. Although copper depressed the rate of growth, volatile acid and CO_2 production after 13 days were approximately equal for all levels

TABLE 4

The effects of Cu in experimental Swiss cheese

	I	II	III
		Analyses	
Copper (*ppm.*)	0.0–0.5	7.5–8.0	18.0
Moisture (%)	38.1	37.7	37.1
pH (7 d.)	5.35	5.30	5.25
(14 d.)	5.48	5.45	5.30
(37 d.)	6.00	5.90	6.00
(88 d.)	6.15	6.00	.....
Relative volatile acidity (*ml. 0.1 N NaOH*) (88 d.)	37.6	41.7	8.6
Cheese characteristics (88 d.)			
Body texture	Good	Good	Poor
Flavor	Good	Excellent	Poor
Eye formation	Good	Good	Poor

of copper studied. Copper did not alter the ratio of acetic to propionic in the volatile acid fraction.

Preliminary evidence indicated that high levels of copper (18.0 ppm.) in Swiss-type cheese exerted an unfavorable influence on the course of ripening, whereas a normal and satisfactory ripening occurred in cheeses containing 0 to 0.5 and 7.5 to 8.0 ppm. copper.

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A TEST OF THE HEAT STABILITY OF MILK PROTEINS FOR USE IN RESEARCH[1]

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For research purposes, it is desirable to have a test for the heat stability of the proteins in a milk sample which is not only accurate and reproducible but also will characterize the particular coagulation phenomenon occurring in the sample. Tests currently used for the study of heat stability, while they may vary in details, are based upon visual observation of either the coagulation time at a fixed temperature (1, 4, 5) or the coagulation temperature for a fixed time interval (3). Investigators employing these tests long have recognized that the character of the coagulum varies from sample to sample, but, by the technique employed, were unable to describe this variation quantitatively.

In order to accomplish this characterization of the coagulation phenomena, the procedure described in this paper was developed, based upon the method first suggested by Miller and Sommer (3) and later modified by Cole and Tarassuk (1).

METHOD

The reagents are the same as for any rapid micro-nitrogen determination, such as that of Hetrick and Whitney (2).

Apparatus. (a) Quevenne (U.S.D.A.) lactometer and cylinder. (b) Hypodermic syringe, 1-ml. capacity with a no. 18 needle and accurately calibrated with mercury. (c) Pyrex glass ampules, 12 to 13 cm. long and $\sim$ 6 mm. inside diameter and made from Pyrex glass tubing of 8 mm. outside diameter. (d) Mechanical rocker with constant-speed motor, as illustrated in figures 1 and 2. (e) Paraffin-oil bath, thermostatically controlled to $\pm$ 0.5° C. (f) Thermometer, -10 to 205° C. and calibrated against a Bureau of Standards thermometer. (g) International no. 2 Centrifuge with no. 813 angle head and accessories. (h) Glass funnels, 5 cm. top diameter, short stem. (i) Test tubes, 5 cm. long and 1 cm. diameter. (j) Filter paper, Whatman 41 H, 3 cm. diameter. (k) Pipette, 0.1-ml. capacity, Normax quality. (l) Volumetric flasks, 50-ml. capacity. (m) Apparatus for micro-nitrogen determination.

Procedure. The specific gravity of the milk sample is determined at 15.5° C. with the Quevenne (U.S.D.A.) lactometer and 1.00 ml. is transferred at that temperature to each of nine ampules by means of the hypodermic syringe. The ampules are sealed, and eight of them are placed in the mechanical rocker. During the filling and sealing operation, the ampules are kept in an ice bath. The rocker then is placed in the oil bath thermostatically controlled at the de-

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sired temperature (120° C. in this study) and rocked at the rate of 24 oscillations per minute. The time of immersion in the oil is recorded. At the first indications of incipient coagulation, the time is noted and an ampule removed from the bath to ice water. The rest of the samples are transferred at suitable time intervals thereafter (at 120° C., 3 to 5 min. intervals).

Five min. after the removal of the last sample from the oil bath, all the ampules, including the ninth ampule, which was unheated, are opened and centrifuged at 3,000 r.p.m. for 30 min. The angle and distance from the center of rotation of all the ampules should be uniform during centrifugation. Care

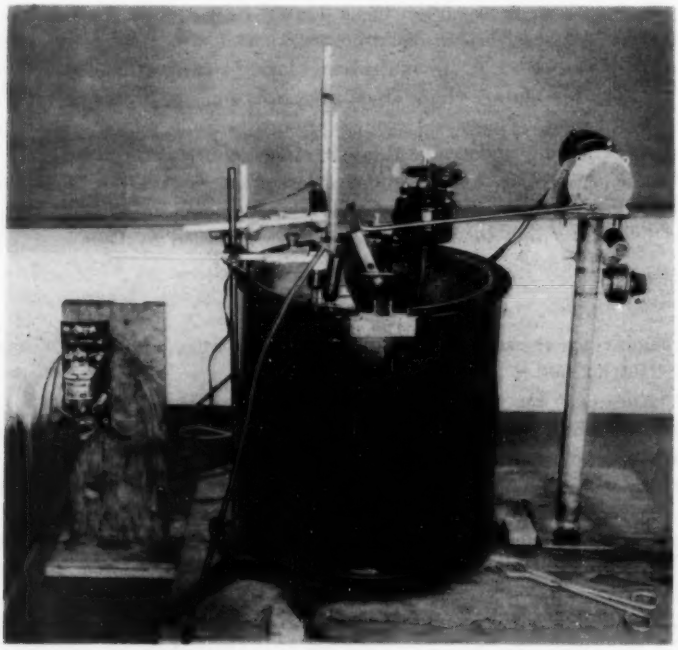

FIG. 1. Heating assembly for heat stability test with mechanical rocker in place.

should be exercised in stopping the centrifuge, since the sedimented material is easily disturbed. The brake should never be used, and the speed should be reduced by a standard procedure.

The supernatant suspensions are decanted carefully into the filters and the filtrates collected in the small test tubes. Ten sec. are allowed for the drainage of the ampules. If fat plugs are present, they must be broken carefully by means of a small glass rod. The filtrates are agitated thoroughly to assure uniformity, and 0.100 ml. of each is transferred to 50-ml. volumetric flasks. The flasks then are filled to the mark with NH_3-free water and analyzed for nitrogen by a micro-nitrogen determination, such as that of Hetrick and Whitney (2). The percentage of filterable nitrogen in each ampule is calculated from the

volume relationships and the specific gravity of the milk and plotted against the time of heating. S-shaped curves are obtained which characterize the milk investigated.

RESULTS

Types of heat stability curves. Throughout this study the milk used was obtained from the University of Illinois herd. While no further attention was paid to the source of the sample, a wide variety of heat stability curves was

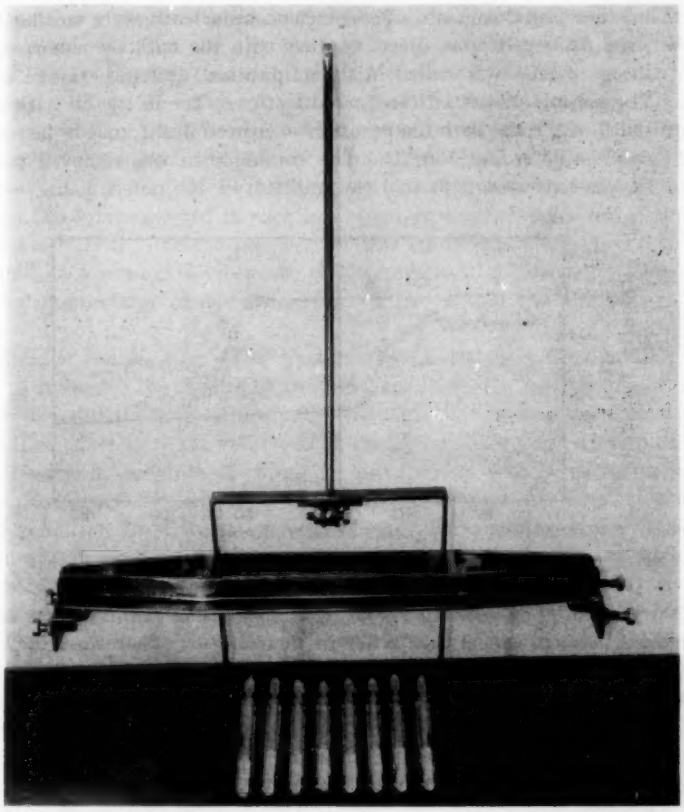

FIG. 2. Mechanical rocker removed from bath showing the ampules of milk

obtained, as illustrated by the three curves in figure 3. The curves may differ from one another in (a) the time at which the sharp drop in the curve occurs, (b) the slope of the steep portion of the curve and (c) the increase in filterable nitrogen just prior to the sharp drop.

In order to obtain as complete a picture of the coagulation phenomenon as possible, curve 1 was secured by a procedure similar to that described previously, except that samples were removed every 5 min. up to 60 min. and at 10-min. in-

tervals thereafter. To accomplish this, three series of eight ampules each were run in succession with the same milk sample.

The time required for the milk to reach bath temperatures. While the work of Miller and Sommer (3) indicated that the milk reached bath temperatures in 85 to 110 sec. in a similar apparatus, it was thought desirable to confirm these results wi.h the assembly employed in this study. A special ampule was constructed with a small well punched in it near one end by means of a carbon pencil. A fine copper-constantan thermocouple (B & S gage no. 30) was sealed into the well with "Sauereisen" cement. The thermocouple leads were insulated from each other and protected from direct contact with the bath by means of tygon tubing. One g. of milk was sealed in the ampule and its temperature adjusted to 0° C. The ampule then was transferred to the rocker in the oil bath and the time required to reach the bath temperature measured and found to be ~64 sec. at 100° C. and ~95 sec. at 160° C. The ampule then was removed to the ice bath and the time necessary to cool the milk to 40° C. noted, being ~11 sec.

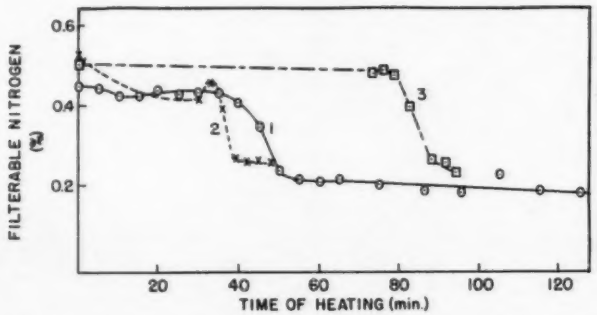

FIG. 3. Examples of heat stability curves obtained from different milks.

from 100° C. and ~31 sec. from 160° C. These time requirements are very short and will tend to cancel each other in their effect. Therefore, they can be ignored with all but the most unstable milks or very high temperatures.

Attempts to preserve the coagulation pattern of the milk. In order to investigate the reproducibility of heat stability curves and the control necessary in the various steps in the procedure, it would be advantageous to preserve the coagulation pattern of the milk sample for a sufficient length of time to perform a suitable number of heat stability curves. Therefore, the following methods for the preservation of the sample against change upon storage were investigated: (a) Storage of the milk sample at ~−25° C. in the frozen state. (b) Storage of the milk sample in lyophilized form (4.7 per cent moisture) at ~5° C. (c) Addition of toluene (0.1 ml. per 100 ml.) to the milk sample and storage at ~5° C. (d) Addition of chloroform (1 ml. per 100 ml.) to the milk sample and storage at ~5° C. (e) Heating the milk sample in a Mallorizer at 148.5° C. for 2.1 sec. with 4 sec. heating and cooling cycles and storage at ~5° C. In all cases, the shapes of the heat stability curves were not only changed by the treat-

ment, as might be expected, but also continued to vary throughout the storage period.

Precision of the test. Since a method for the preservation of the coagulation pattern of the milk sample did not appear to be available, a single point on the steep portion of the heat-stability curve was selected to determine the control necessary in the various steps of the procedure and the reproducibility of the entire curve. This procedure is justified by preliminary observations that this region of the curve is most sensitive to changes in technic. Also, it allows for a sufficient number of replicates for statistical treatment within a reasonably short time interval to minimize the changes occurring in the milk sample. For all subsequent work, skimmilk heated at 148.5° C. in a Mallorizer was employed.

From a consideration of the results of all of the studies performed, some of which are reported in the following tables, the error variance was found to vary from 486×10^{-6} to 2×10^{-6}, which upon application of the χ^2 test indicated some heterogeneity. While proof is lacking, this heterogeneity may be due to the position on the curve selected in each investigation and the behavior of the individual milk samples. For the purposes of this study, a mean value of 95×10^{-6} was selected as a reasonable measure of the precision of the test. This represents an optimum standard deviation of $\pm$ 3.2 per cent of the filterable nitrogen value.

The effect of sample size. The amount of milk sealed in the ampule may be expected to influence the results of the test since both the motion of the sample in the ampule during heating and the sedimentation phenomenon in the centrifuge will be affected by the volume of the sample. Therefore, 24 ampules were filled with varying amounts of skimmilk and divided into three series so that each series contained duplicates of each of the sample sizes. They were heated for a sufficient time to be in the steep portion of the coagulation curve, as indicated by a preliminary run, and treated according to the indicated method. The experiment was repeated a few days later and the combined results are reported in table 1. Analysis of variance of these results indicates that the effect of size of sample varies with the condition of the milk at the time of testing (days and series interaction terms are significant). Therefore, careful control of the sample size is indicated. However, with care the tolerance of sample size can be maintained at $\pm$ 0.01 which may be expected to contribute 1×10^{-6} of the error variance of 95×10^{-6}.

The effect of diameter of the ampule. The diameter of the ampule employed may influence the results of the test in a manner similar to the effect of the size of the sample. Therefore, 1.00 ml. of mallorized skimmilk was sealed in each of 24 ampules of measured inside diameters varying from 5.50 to 6.25 mm. The ampules were divided into three series each covering the range of diameters, heated for the appropriate time in the oil bath and then treated according to the usual procedure. The experiment was repeated with another milk and the combined results are reported in table 2. Analysis of covariance indicated a significant regression of filterable nitrogen on ampule diameter. This regression coefficient is sufficiently small, however, to make the usual extreme variation en-

countered in the glass tubing ($\pm$ 0.5 mm.) acceptable since this would contribute only 19×10^{-6} of the error variance of 95×10^{-6}.

The effect of the temperature of the oil bath. In order to determine the effect of small variations in the temperature of the bath, three series of eight samples each were heated for 100 min. in the oil bath at 118.5, 120 and 121.5° C., respectively, and cooled, centrifuged, filtered and analyzed as before. The results are presented in table 3. Analysis of variance of these results indicates a very significant linear response with temperature and a questionable quadratic response. For purposes of control, it is sufficient to consider the linear regression coefficient. For this temperature range, therefore, the expected mean tem-

TABLE 1

The effect of size of milk sample upon the filterable nitrogen content of partially heat-coagulated skimmilk

Sample size (*ml.*)	Average filterable nitrogen (%) (Duplicates)					
	1st day			2nd day		
	Series 1	Series 2	Series 3	Series 1	Series 2	Series 3
0.8	0.382	0.388	0.364	0.340	0.344	0.328
0.9	0.362	0.348	0.378	0.324	0.317	0.324
1.0	0.394	0.414	0.400	0.314	0.329	0.314
1.1	0.390	0.386	0.398	0.340	0.362	0.360

Analysis of variance

Source of variance	Degrees of freedom	Sum of squares $\times 10^6$	Mean square $\times 10^6$	Variance ratio	Significance
Days (D)	1	31,008	31,008	20.98	5%
Series (S)	2	195	98	0.241	n.s.
Sample size (T)	3	5,602	1,867	1.263	n.s.
D $\times$ S	2	144	72	1.059	n.s.
D $\times$ T	3	4,435	1,478	21.735	0.1%
S $\times$ T	6	2,438	406	5.971	0.1%
D $\times$ S $\times$ T	6	578	96	1.412	n.s.
Error	24	1,631	68		
Total	47	46,031			

perature control of $\pm$ 0.1° C. would contribute only $\sim 1 \times 10^{-6}$ of the error variance of 95×10^{-6}. However, a known temperature variation of 0.2° C. would induce a relative error of ~ 2 per cent in the filterable nitrogen value.

The effect of the speed of the rocker. The speed of the rocker is reported by Cole and Tarassuk (3) to influence the results of the experiment. In order to determine the magnitude of this effect and the control necessary, three series of eight samples each were heated for the appropriate time interval in the oil bath at rocker speeds of 19, 24 and 36 complete swings per minute and then processed in the usual way. The experiment was repeated upon a different milk and the combined results reported in table 4. Analysis of variance of these results indicates that within the range investigated, the filterable nitrogen content is independent of the speed of the rocker.

The effect of time of holding in the ice bath after heating. Since the time of

TABLE 2

The effect of the diameter of the ampules upon the filterable nitrogen content of partially heat-coagulated skimmilk

Milk	Series 1		Series 2		Series 3	
	Diameter of ampule	Filterable nitrogen	Diameter of ampule	Filterable nitrogen	Diameter of ampule	Filterable nitrogen
	(*mm.*)	(%)	(*mm.*)	(%)	(*mm.*)	(%)
1	5.50	0.391	5.50	0.417	5.60	0.396
	5.70	0.392	5.60	0.405	5.65	0.394
	5.85	0.388	5.70	0.418	5.85	0.411
	5.90	0.399	5.85	0.423	5.95	0.409
	6.00	0.402	5.95		6.00	0.402
	6.10	0.405	6.00	0.418	6.05	0.420
	6.20	0.414	6.15	0.432	6.10	0.417
	6.25	0.414	6.20	0.431	6.20	0.429
2	5.50	0.298	5.55	0.303	5.55	0.303
	5.60	0.341	5.65	0.323	5.80	0.311
	5.85	0.343	5.75	0.291	5.85	0.309
	5.90	0.330	5.90	0.323	5.95	0.319
	6.00	0.344	6.00	0.313	6.05	0.311
	6.10	0.348	6.10	0.329	6.10	0.309
	6.15	0.363	6.15	0.317	6.15	0.317
	6.25	0.328	6.20	0.313	6.20	0.310

Analysis of covariance

Source of covariance	d. f.	$Sx^2 \times 10^4$	$Sxy \times 10^5$	$Sy^2 \times 10^6$	Adj. d. f.	Adj. $Sy^2 \times 10^6$	M.S. $\times 10^6$	V.R.	Sig.
Total	46	24,728	4,261	121,648	45	120,914			
Error	41	24,316	7,283	5,650	40	3,469	86.7		
Series	5				5	117,445	23,489	271	0.1%
Regression					1	2,181	2,181	25.2	0.1%

Regression coefficient = 0.0300 ± 0.0060% N/mm.

holding in the ice bath after heating might influence the results of the test by permitting further aggregation of the denatured proteins, its effect was investigated. Three series of eight samples each of skimmilk were heated for 45 min.

TABLE 3

The effect of temperature upon the filterable nitrogen content of partially heat-coagulated skimmilk

Temperature (° *C.*)	118.5	120.0	121.5
No. of ampules	8	8	8
Average filterable nitrogen (%)	0.396	0.334	0.299

Analysis of variance

Source of variance	Degrees of freedom	Sum of squares $\times 10^6$	Mean square $\times 10^6$	Variance ratio	Significance
Temperature					
Linear response	1	37,830	37,830	221.22	0.1%
Quad. response	1	1,027	1,027	6.01	5%
Error	21	3,592	171		
Total	23	42,449			

Linear regression coefficient = -0.0323 ± 0.0022% N/° C.

TABLE 4

The effect of the speed of rocking upon the filterable nitrogen content of partially heat-coagulated skimmilk

Oscillations/ min.	Milk 1		Milk 2	
	No. of ampules	Av. filterable nitrogen	No. of ampules	Av. filterable nitrogen
		(%)		(%)
19	8	0.251	8	0.472
24	7	0.246	8	0.469
30	8	0.246	8	0.462

Tests of significance

Source of variance	Degrees of freedom	Sum of squares $\times 10^6$	Mean square $\times 10^6$	Variance ratio	Significance
Error	41	3,364	82		
Interaction (free of milks & speeds)	2	175	88	1.07	n.s.
Speed of rocking (free of milks & interaction)	2	403	202	2.47	n.s.
Milks (free of speeds & interaction)	1	564,742	564,742	6,880	0.1%

in the oil bath. Each series was divided into four pairs of samples which were held for 5, 40, 75 and 110 min., respectively, in the ice bath, centrifuged, filtered and analyzed in the usual manner. The results are reported in table 5. Analysis of variance of the results indicates a possibility of a linear holding-time effect. However, the linear regression coefficient is sufficiently small that the error intro-

TABLE 5

The effect of holding time in the ice bath upon the filterable nitrogen content of partially heat-coagulated skimmilk

Holding time in ice bath	Average filterable nitrogen (%) (Duplicates)		
	Series 1	Series 2	Series 3
(*min.*)			
5	0.336	0.374	0.394
40	0.351	0.362	0.400
75	0.352	0.372	0.384
110	0.366	0.380	0.398

Analysis of variance

Source of variance	Degrees of freedom	Sum of squares $\times 10^6$	Mean square $\times 10^6$	Variance ratio	Significance
Series (S)	2	7,268	3,634	51.26	0.1%
Holding time (T)					
Linear response	1	488	488	6.88	5%
Quadratic response	1	122	122	1.72	n.s.
Cubic response	1	108	108	1.52	n.s.
S × T	6	949	158	2.23	n.s.
Error	12	851	71		
Total	23	9,786			

Linear regression coefficient = 0.000115 ± 0.000043% N/min.

duced from this source will not contribute more than $\sim 2 \times 10^{-6}$ of the error variance of 95×10^{-6} in 40 min., the extreme difference in holding time between ampules in the same test. However, a difference in holding time greater than 50 min. will introduce a relative error in the filterable nitrogen of greater than 2 per cent.

The effect of centrifugal speed. In order to determine the most suitable centrifuge speed and the control necessary for this variable, two different experiments were performed. In the first experiment, unheated skimmilk was centrifuged in duplicate ampules, each containing 1.0 ml. of sample for 30 min. at six different centrifuge speeds, filtered and analyzed as indicated in the procedure. The results are shown in table 6. Analysis of variance of these results

TABLE 6

The effect of centrifuge speed upon the filterable nitrogen content of unheated skimmilk

Speed of centrifugation (*r.p.m.*)	0	1,000	1,500	2,000	2,500	3,000	3,500
Average filterable nitrogen (%) (Duplicates)	0.567	0.563	0.560	0.559	0.560	0.556	0.538

Analysis of variance

Source of variance	Degrees of freedom	Sum of squares $\times 10^6$	Mean square $\times 10^6$	Variance ratio	Significance
Speed					
Linear response	1	669	669	25.05	0.1%
Quadratic response	1	140	140	5.24	n.s.
Remainder	4	196	49	1.84	n.s.
Error	7	187	26.7		
Total	13	1,192			

Linear regression coefficient = $1.43 \pm 0.41 \times 10^{-6}$% N/r.p.m.

indicates a significant linear regression, but the regression coefficient is so small that less than 1 per cent of the nitrogen content of the sample would be sedimented at 3,000 r.p.m., which could not be detected by the test when performed according to the procedure.

In the second experiment, three series of eight samples each of mallorized skimmilk were heated for 48 min. at 120° C. and cooled. One pair of ampules from each series was centrifuged for 30 min. at each of the following speeds: 2,000, 2,500, 3,000 and 3,500 r.p.m., filtered and analyzed as before. The results are given in table 7. As would be expected, the amount of sedimented protein is a function of the speed of sedimentation and analysis of variance indicates this to be a linear response if the one pair of values obtained in the third series at 2,500 r.p.m. is ignored. However, this pair does contribute significant curvature to the relationship for that series. Observations on the variation of the centrifuge speed with time and repeated runs indicated that $\pm$ 50 r.p.m. is the limit of control of the centrifuge speed obtainable without further modification. This control is sufficient, since it will contribute only $\sim 3 \times 10^{-6}$ of the error

variance of 95×10^{-6}. However, known errors in centrifuge speed greater than this will introduce relative errors in the filterable nitrogen value of ± 2 per cent or greater.

The effect of time of centrifugation. The most suitable time of centrifugation and the necessary control also were studied. Three series of eight samples each of mallorized skimmilk were heated for 67 min. at 120° C. and cooled. Two samples of each series and an ampule containing the unheated milk then were centrifuged for 10, 25, 30 and 35 min., respectively, and treated as before. The results are shown in table 8. A very gradual decrease in filterable nitrogen can be observed in the unheated milk but it remains within the 95 per cent confidence limits for the first 30 min. As was expected, the filterable nitrogen obtained

TABLE 7

The effect of centrifugal speed upon the filterable nitrogen content of partially heat-coagulated skimmilk

Speed of centrifugation	Average filterable nitrogen (%) (Duplicates)		
	Series 1	Series 2	Series 3
(*r.p.m.*)			
2,000	0.544	0.523	0.531
2,500	0.494	0.498	0.452
3,000	0.422	0.412	0.418
3,500	0.352	0.366	0.359

Analysis of variance

Source of variance	Degrees of freedom	Sum of squares $\times 10^6$	Mean square $\times 10^6$	Variance ratio	Significance
Series (S)	2	748	374	.....	n.s.
Centrifugal speed (C)					
Linear response	1	102,726	102,726	236.7	0.1%
Quad. response	1	68	68	.....	n.s.
Cubic response	1	88	88	.....	n.s.
S × C	6	2,607	434	6.00	1%
Error	12	868	72		
Total	23	107,100			

Linear regression coefficient = 0.000116% N/r.p.m.

from the partially heat-coagulated skimmilk is dependent upon the time of centrifugation but is approaching equilibrium after 30 min. "Student's t" for the observed difference between 30 and 35 min., is significant at the 3 per cent level, but this may be due to sedimentation of uncoagulated proteins, since this difference is approximately the same as that observed in the unheated milk. Therefore, centrifuging longer than 30 min. does not seem desirable. Consideration of the last three times of centrifugation indicates a linear regression coefficient of -0.00448 per cent N per minute. If the centrifuge time is controlled to ± 1 min., the error introduced from this source will not contribute more than $\sim 2 \times 10^{-6}$ of the error variance of 95×10^{-6} but a known error of ± 1.5 min. would introduce a relative error in the filterable nitrogen of ± 2 per cent.

The effect of type of filter paper. Several different grades of filter paper

were investigated and the Whatman 41 H paper was found most suitable. It is very rapid and therefore loss of water from the filtrate by evaporation is kept at a minimum. It is not intended that the filter paper should retain particles too small to sediment completely, but rather it is employed as an added precaution against contamination of the filtrate by dislodged pieces of the sedimented material during decantation.

TABLE 8

The effect of time of centrifugation upon the filterable nitrogen content of unheated and partially heat-coagulated skimmilk

Time of centrifugation	Average filterable nitrogen (%) (Duplicates)				
	Unheated skimmilk	Partially heat-coagulated skimmilk			
		Series 1	Series 2	Series 3	Mean
(*min.*)					
10	0.548	0.436	0.450	0.443	0.443
25	0.536	0.270	0.299	0.323	0.297
30	0.533	0.252	0.278	0.276	0.269
35	0.516	0.247	0.257	0.254	0.253

Analysis of variance

Source of variance	Degrees of freedom	Sum of squares $\times 10^6$	Mean square $\times 10^6$	Variance ratio	Significance
Series (S)	2	24	1,211	10.91	1%
Time of centrifugation (T)	3	135,796	45,265	407.8	0.1%
S × T	6	1,392	232	2.09	n.s.
Error	12	1,336	111		
Total	23	140,946			

DISCUSSION

Contrary to the tests currently available, this test does not supply a single value for the coagulation time but rather a more complete picture of the coagulation phenomena in the critical time interval. If a single value is desired for use in studies relating coagulation time to some other property of the milk, some unique point on the curve could be selected for this purpose, such as the point of inflection of the curve or the point at which a certain per cent of the total nitrogen is sedimented. This procedure would be as valid as those now employed.

The significance of the different shapes of the curves obtained has not been established. However, some speculation at this time seems justified. The nitrogen value obtained at each point on the curve consists of five different classes of nitrogenous material: (a) the non-protein nitrogen unaffected by heat, (b) the unchanged proteins originally present in the milk, (c) the denatured but uncoagulated proteins, (d) the coagulated proteins of a size sufficiently small to be incompletely sedimented and (e) decomposition products of the protein due to heating.

The non-protein nitrogen unaffected by heat will remain constant throughout any one curve. The unchanged proteins in the milk as time progresses will be-

come denatured according to their own rates. This process undoubtedly is proceeding throughout the initial part of the curve and group (b) is converted to group (c) without any observable effect on the test.

As the denaturation proceeds, changes of a sufficient magnitude are produced in the charge and degree of hydration such that coagulation can take place. The sizes of particles formed over any time interval will be distributed over a range of values dependent upon the probability of collision of particles of the required sizes and the probability of adhesion upon collision. The probability of adhesion will be dependent upon the extent of the change in the degree of hydration or charge of the colliding particles. This may be expected to vary with the milk under consideration. Therefore, if the motion of the milk is sufficient to keep the probability of collision high compared to the probability of adhesion, the shape of the curves will be dependent upon the rate of change of the degree of hydration or charge of the denatured particles, since they will determine the size distribution of the coagulated particles. Superimposed upon these phenomena will be the decomposition phenomena, which are proceeding throughout the entire heat treatment according to their various rates.

While the difficulties involved are great due to the complexity of the problem, it is hoped that a semi-quantitative treatment along the lines of these hypotheses may yield information as to the various rate constants involved and, therefore, open the way for the investigation of the effects of different variables upon the rate constants in the natural milk environment.

SUMMARY

A test of the heat stability of milk proteins has been described which is suitable for research purposes. The necessary control of the different variables in the test has been evaluated and the reproducibility of the test established. The possible significance of the results obtained from the test was briefly discussed.

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EFFECTS OF FEEDING LARGE AMOUNTS OF GRASS SILAGE TO DAIRY BULLS ON FEED UTILIZATION, BODY MEASUREMENTS AND SEMEN QUALITY[1]

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Silage, either corn, grass or legume, is fed in only about 20 per cent of the artificial breeding associations in the country, according to a 1949 survey (1). In the reporting associations the average daily silage consumption was 16 lb. per bull. While reluctance to feed larger amounts of silage does not appear to be based upon controlled experiments, early recommendations (4, 8) restricted silage intake to 15 lb. daily in order to avoid paunchiness and/or slowness at service. More recent work (7, 12) has shown that moderate amounts of grass silage may be fed without impairing the libido or fertility of the bull. In contrast to this limited information on silage for bulls, investigators (2, 3, 5, 7, 11) have demonstrated that silage may safely constitute a major portion of the rations of milk-producing cows and growing dairy heifers. Obviously research on roughage feeding of dairy bulls has lagged behind the rapid development of the modern concept of grassland farming.

The present investigation was undertaken to determine what effect, if any, the feeding of large amounts of grass silage has upon the body measurements of the bull and upon the quality and quantity of the semen produced.

EXPERIMENTAL

Selection of animals. Twelve dairy bulls were selected and paired with respect to breed, age, body weight and quality of semen produced. Of each pair, one bull was selected at random and assigned to the silage group, while his pair mate was placed in the control group. Six Holsteins, four Guernseys and two Jerseys, ranging in age from 1.7 to 9.8 yr., were paired and assigned to groups as indicated in table 1. Pair mates were well matched with the exception of the two Jerseys, J43 and J34, for which the difference in age was large.

Plan of experiment. During the experimental period, each silage-fed bull received a ration of grass-legume silage at the rate of 4 lb. per 100 lb. of body weight per day, plus sufficient hay to bring the total digestible nutrient (TDN) intake up to the recommended allowances of the National Research Council (9). Each control bull received the same amount of hay as his pair mate on silage, plus a concentrate mixture in place of and calculated to be equal to the TDN content of the silage. Thus, a 2,000 lb. bull received 80 lb. of silage and 8 lb. of hay if on the all-roughage ration, or 8 lb. of hay and 15 lb. of concentrate if on the control ration. While this is more concentrate than commonly is fed to bulls, it was

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deemed desirable to alter only the proportion of silage and concentrate fed the two groups, rather than to introduce the added variable of a changing hay intake.

The trial consisted of three phases: a 4-wk. pre-experimental period, an experimental period consisting of 13 4-wk. periods or months, and a post-experimental period of 8 wk. in which the silage-fed bulls were observed for any possible changes after being returned to a hay-concentrate ration. Due to space commitments for other experiments, it was impossible to carry the control bulls through the post-experimental period.

Prior to the initiation of the trial and during the pre-experimental period, Holstein bulls received a daily allotment of 15 to 18 lb. of hay and 4 lb. of concentrate, while Guernseys and Jerseys were given 12 to 15 lb. of hay and 3 lb. of

TABLE 1

TDN consumption and changes in body measurements in bulls over an experimental period of 52 wk.

Bull	Group	Age	Av. daily TDN consumed	Total gain in			
				Wt.	Heart girth	Paunch girth	Ht.
		(*yr.*)	(*lb.*)	(*lb.*)	(*in.*)	(*in.*)	(*cm.*)
G33	G	4.5	11.3	49	−2.8	3.5	1.5
G36	S	4.8	10.4	219	0.8	10.5	2.0
G35	G	7.3	10.6	−15	−2.5	−3.4	0.0
G37	S	5.5	8.3	39	−3.9	−0.5	−0.5
H40	G	1.7	13.1	517	6.0	6.1	11.0
H38	S	1.7	12.4	525	6.5	9.9	15.0
H39	G	1.7	14.0	543	6.3	8.6	13.5
H42	S	1.7	13.6	585	7.5	8.4	13.5
H41	G	3.8	14.8	232	0.5	4.8	0.0
H44	S	3.1	14.4	459	3.5	9.4	0.0
J43	G	9.8	11.2	78	−2.8	0.8	2.0
J34	S	2.3	10.2	305	1.5	9.4	4.0
Av. grain			12.5	234 ± 99[a]	0.8 ± 1.8	3.4 ± 1.7	4.7 ± 2.4
Av. silage			11.5	355 ± 84	2.6 ± 1.7	7.8 ± 1.7	5.7 ± 2.8

[a] Standard error of the mean.

concentrate. Throughout the trial two successive ejaculates of semen were collected on a weekly schedule. Semen was evaluated on the basis of laboratory tests, which included volume, concentration, initial motility, livability, unstained (live) sperm and acid and alkaline phosphatase. Concentration of spermatozoa was determined with the hemacytometer. Livability was measured by estimating the per cent of motile spermatozoa at 37° C. following storage at 4° C. for 5, 10 and 15 days. The semen aliquots used for storage were diluted 1:50 in egg yolk-citrate. The percentage of unstained or live spermatozoa was determined by the method of Shaffer and Almquist (14). A modification of the Gutman and Gutman procedure (6) was used for phosphatases. Citrate buffer was used for acid phosphatase (pH 4.9), while sodium barbitol solution was used as the buffer for alkaline phosphatase (pH 9.0). The substrate in each case was disodium phenyl phosphate.

Bulls were rated for libido at the time of collection on a scale ranging from zero for refusal to serve to three for "very active" service.

Feeding and management. Bulls were weighed weekly; heart girth and paunch girth were measured every 2 wk. and height at withers every 4 wk. The concentrate fed, a commercial mixture widely used for bull feeding in Pennsylvania, contained several cereal grains and protein supplements and was fortified with trace minerals and vitamins A and D. Blocks of trace-mineralized salt were available to all bulls. The hays fed were timothy-clover mixtures containing from 60 to 80 per cent timothy. The silage fed the first 7 mo. was 1950 crop timothy-clover with 200 lb. of oat mill feed per ton of green material added as a preservative. Silage fed the latter part of the trial was 1951 crop timothy-clover preserved with 7 lb. SO_2 per ton of green material (15). Crude protein content of the concentrate, hay and silage averaged 15.0, 8.2 and 4.1 per cent, respectively. The average moisture content of the silage as fed was 70.4 per cent. Rations were calculated on the basis of TDN values recommended by Morrison (10).

RESULTS

Body measurements and feed utilization. The control bulls consumed an average of 7.6 lb. of hay and 11.6 lb. of grain (16.9 lb. of dry matter) per day, compared to an average daily consumption of 7.3 lb. of hay and 56.8 lb. of silage (22.9 lb. of dry matter) for the silage-fed bulls. Additional data pertaining to nutrient consumption and changes in the body measurements of individual bulls are presented in table 1. In each case the figure listed as total gain is the difference between the average value for the 13th experimental 4-wk. period and the average for the pre-experimental period. It will be noted within each pair that the TDN consumption was lower for the silage-fed than for the grain-fed bull. This was due principally to the occasional refusal of silage accompanying the high rate of silage feeding. Although during most of the trial there was very little feed refusal, there were times, such as at the beginning of the experimental period and again at the time the 1951 crop of silage was introduced, when silage weighbacks were considerable. Despite the apparent lower nutrient intake, each silage-fed bull gained more weight than did his pair mate on concentrate. Thus, it appears that either the nutritive value of the silage was underestimated or the bulls were better able to utilize their feed on the high-silage ration. Similar results with cows have been reported by Henderson and Norton (7). While the silage-fed bulls gained considerably more in weight, hearth girth, paunch girth and height at withers than did control bulls, the differences did not reach statistical significance at the five per cent level when tested by the t test (16).

Additional information on body measurements is presented in figure 1, which gives the monthly averages for each of the body measurements and for daily TDN consumption. Obviously much of the final difference noted between treatments was evident within the first month. The drop in TDN consumption for the experimental group at 5 and 6 mo. is believed attributable to the poor quality hay available at that time. The TDN consumption increased when good quality hay was provided during the seventh month, but declined again the eighth

month when a new crop of silage was introduced. The experimental bulls obtained adequate roughage in the silage and refused some of the hay, whereas the control bulls on the restricted roughage ration ate the hay, even though it was of poor quality.

Semen characteristics. Monthly trends in semen volume, concentration of spermatozoa, unstained (live) spermatozoa and acid and alkaline phosphatases are shown in figure 2. Although there were variations from month to month,

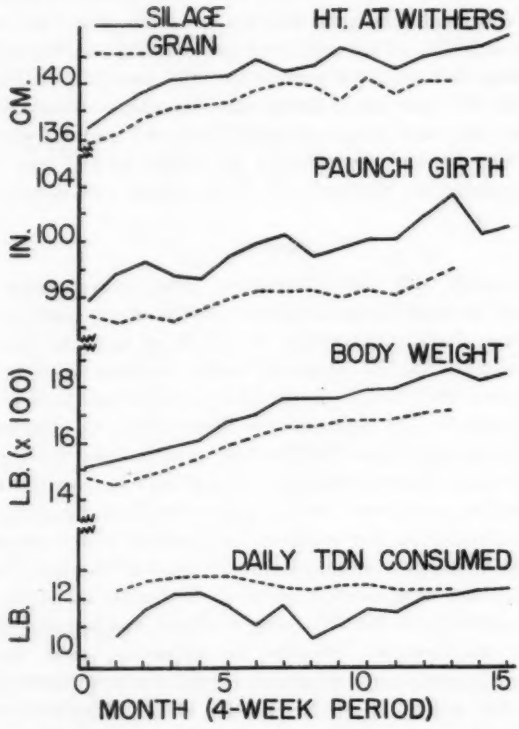

FIG. 1. Monthly averages of body measurements and daily TDN consumption of bulls fed the silage-hay versus the grain-hay ration.

particularly in concentration, silage feeding had neither a beneficial nor a detrimental effect upon these characteristics. The average values for the experimental period for silage-fed and control bulls, respectively, were volume, 4.8 and 4.8 ml.; concentration, 135×10^7 and 133×10^7 sperm per milliliter; unstained (live) spermatozoa, 66 and 67 per cent; acid phosphatase, 6.0 and 7.3 Gutman units per milliliter, and alkaline phosphatase, 5.7 and 4.6 Gutman units per milliliter. There was a tendency for semen from silage-fed bulls to be slightly higher in alkaline phosphatase and slightly lower in acid phosphatase than semen from control bulls.

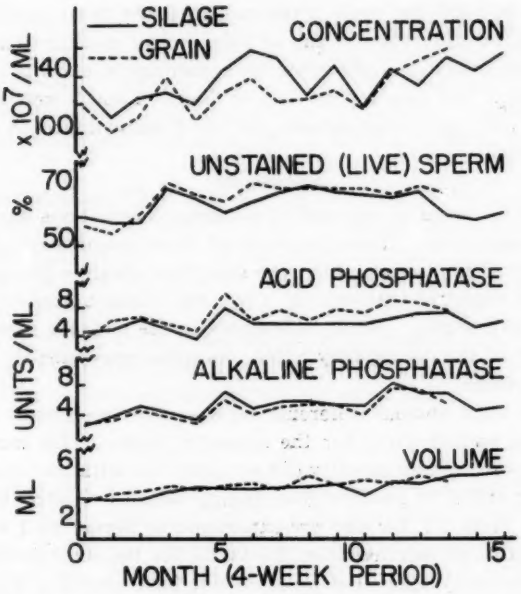

FIG. 2. Monthly averages for volume, concentration, unstained (live) spermatozoa and acid and alkaline phosphatases of semen from bulls fed the silage-hay versus the grain-hay ration.

Presented in figure 3 are the curves for the initial motility of the spermatozoa, and the motilities after 5, 10 and 15 days of storage at 4° C. Motilities, both initial and after storage, were low the first month of the experiment for both

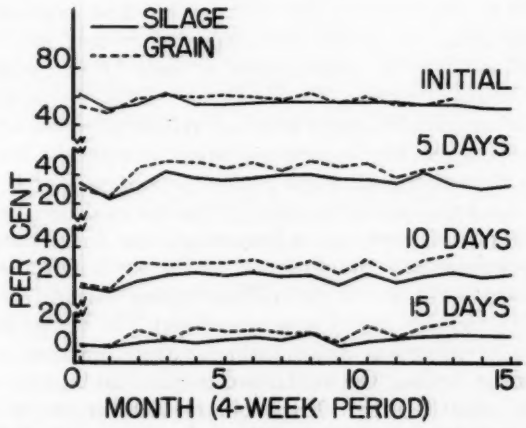

FIG. 3. Monthly averages of initial motility of spermatozoa and motility after storage of semen from bulls fed the silage-hay versus the grain-hay ration.

groups of bulls and did not reach a plateau until the third month of the trial. While there was no difference in the average initial motility, the spermatozoa from control bulls showed a slight apparent advantage in motility after 5, 10 and 15 days of storage. All data represented in figures 2 and 3 were tested by analysis of variance (16) and in no instance did the *F* value approach significance at the 5 per cent level.

DISCUSSION

In this study the levels of acid and of alkaline phosphatases were of about the same order of magnitude. Thus, the ratio of these constituents does not agree with the findings of Reid *et al.* (13), who state that alkaline phosphatase is predominant, or the report of Quicke *et al.* (12), who claim a predominance of acid phosphatase in bull semen. While differences in the methods used undoubtedly account for some of the discrepancy noted, phosphatases evidently are extremely variable in bull semen.

Probably the most obvious difference between the two groups is the greater gain (4.4 in.) in paunch girth for the silage-fed bulls. This increase was not detectable to the eye, and apparently did not interfere with the sexual activity of the bulls or their ability to produce good quality semen. Control bulls decreased in libido rating from 2.1 for the pre-experimental period to 1.9 for the 13th month, compared to an increase from 2.4 to 2.5 for the same period for experimental bulls. Actually silage-fed bulls should have shown a greater increase in paunch girth than the control bulls, since their increase in body weight was greater. In reality the difference between the 13th month and the pre-experimental month may not present a true picture of the results obtained. All measurements made during the experimental period, when compared to the pre-experimental period, were reduced in magnitude for control bulls due to the reduction in fill (increase in concentrate and decrease in hay) but were increased for experimental bulls due to the bulk and weight of the silage. To avoid this bias, a comparison could be made between the 13th and the first experimental months. On this basis the gains for control and silage-fed groups, respectively, were: Body weight, 255 and 330 lb.; hearth girth, 0.7 and 1.9 in.; paunch girth, 4.0 and 5.8 in.; and height at withers, 3.9 and 4.4 cm. Thus, the increase in paunch girth on silage as compared to grain does not appear to be out of line with the gains made in body weight, hearth girth and height at withers. The reduction in body weight and in paunch girth which resulted immediately after returning the silage-fed bulls to a hay-concentrate ration is further evidence that a large portion of the gain in paunch girth and in body weight was due to the silage fill.

These results substantiate the report of Quicke *et al.* (12), who fed up to 45 lb. of grass silage daily for 12 mo. without noting harmful effects upon the bull or the quality and fertility of semen produced. In the present study this level of grass silage feeding was almost doubled without apparent adverse effects. A high level of silage feeding was established in this trial in order to reveal any possible effects of silage feeding. The large amounts fed (80 lb. of silage and 8 lb. of concentrate daily for a 2,000 lb. bull) indicate the bull's ability to handle quantities of bulky feeds without interfering with his activity. In this trial

silage was fed to bulls up to 6 yr. of age and Quicke *et al.* (12) included 11-yr.-old bulls in their study, but the effects of heavy silage feeding upon older bulls have not been determined. This report and those from Wisconsin (12) and Rhode Island (7) have dealt only with grass silage; the effects of corn silage on dairy bulls should be investigated before a general recommendation on silage feeding can be made.

The economy of silage feeding is difficult to evaluate due to price fluctuations from time to time and from area to area. However, using prices prevalent in Central Pennsylvania at the time of the study, approximately a 20 per cent reduction in feed cost, or a saving of 50 dollars per bull per year, could be effected by feeding the silage ration in preference to a conventional hay-concentrate ration. While shifts in price relationships could narrow this margin considerably, it appears that under most conditions the silage ration would be cheaper than conventional hay-concentrate rations for dairy bulls.

SUMMARY AND CONCLUSIONS

Twelve bulls were paired and divided into two groups, one bull of each pair receiving 4 lb. of silage daily per 100 lb. of body weight and the other receiving an almost equal amount of TDN in concentrates. Hay intake was the same for each member of a pair.

After the 52-wk. trial, the silage-fed bulls had gained more in body weight, heart girth, paunch girth and height at withers than had the concentrate-fed bulls, but the differences between the groups were not significant. Silage-fed bulls apparently made more efficient use of the feed consumed than did concentrate-fed bulls, as shown by larger gains in body weight on smaller TDN consumption. Under existing conditions, the high silage ration offered considerable economic saving over conventional hay-concentrate rations for bulls.

Semen quantity and quality tests were conducted on a weekly basis and showed no statistically significant differences between the two groups.

On the basis of these results, it appears that bulls may be fed grass silage at rates up to 4 lb. per 100 lb. of body weight per day without harmful effects upon the physical condition of the bull or the quantity and quality of semen produced.

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RELATIONSHIP BETWEEN EARLY RATE OF GROWTH AND BUTTERFAT PRODUCTION IN DAIRY CATTLE[1]

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Size of dairy cattle as measured by body weight shows a positive and significant correlation with butterfat production (4, 8). However, Touchberry (14) found that milk and fat production seemed to be genetically independent of body measurements and weight. Since size is the ultimate result of growth and since growth as well as milk and butterfat production is determined partly by the secretions of the same glands, the rate of growth might be associated with potential for milk and butterfat production. Prentice (12) did find that calves with a high efficiency rating for the first 12-mo. gain in weight also turned out to be good producers, whereas calves with a low efficiency rating for gain never proved to be high producers. Bonnier and Hansson (2) concluded that dairy animals have a genetically determined capacity for growth. However, Davis and Willet (5) did not find any relationship between growth and later production when growth was measured as the percentage increase in weight, height at withers and chest girth from birth to 2 yr. of age. Brody (3, p. 497) has shown how the postnatal growth curve may be divided into two principal segments, the first of increasing slope and the second of decreasing slope. According to Brody (3, p. 500) the point of inflection of the growth curve is the point of maximum velocity of growth which in dairy animals is reached at about 6 mo. of age.

The present paper presents the results of a study of the relationship between growth in heart girth and wither heights from 10 to 12 mo. of age and later butterfat production.

MATERIALS AND METHODS

From the University of Nebraska dairy herd, records were available on 105 cows that had been measured at monthly intervals during their early life and which later had at least one complete 305-day record. Of these 105 cows, 78 were Holsteins and 27 were Guernseys with a total of 241 lactation records or an average of 2.30 per cow. Records and measurement data also were available for 33 Jersey cows with a total of 64 records. Bartlett's test for homogeneity of variance as given by Snedecor (13, p. 249) showed, however, that the variance of the records of the Jersey cows was significantly smaller than the variances for the Holstein and Guernsey records. Therefore, in the final computations, the Jersey data were omitted, although the inclusion of these data would not have changed the over-all results.

The rate of growth was calculated as the mean daily increase in height at withers and chest girth from 10 to 12 mo. of age. It would have been more log-

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ical to use the increase in wither height and chest girth at about 6 mo. of age where the velocity of growth is at its maximum, but the errors of measurement of growth seemed to be larger at that age. All production records were calculated as mature equivalent butterfat production for 305 days and were converted to twice-a-day milking for those records that actually were made under three- and four-time milking. The average lifetime production was calculated for each cow, using all records that were at least 305 days in length.

Table 1 shows the averages for the first, second and third records of the Holstein cows that had one, two, three or more records. The figures show that cows with only one record had a lower lifetime average than did cows with more than one record and indicate that some selection on the basis of the first record has taken place in the University dairy herd. For this reason, the most probable producing ability rather than the actual production was used as a measure of producing ability.

TABLE 1

Mean mature equivalent 305 d., 2-time records for 78 Holstein cows

No. of cows	No. of records/ cow	Mean of record no.			Mean of all records
		1	2	3	
24	1	502.8	.....	.....	502.8
27	2	533.6	489.7	.....	511.6
15	3	568.0	514.9	468.7	517.2
12	more than 3	550.5	545.1	568.4	545.4

The most probable producing ability was calculated as the deviation from the mean production of the respective breed in the University herd multiplied by $\frac{N\,r}{1+(N-1)\,r}$ where N is the number of records involved in the average and r is the intrabreed repeatability or correlation between single records of the same cow.

RESULTS

The intrabreed repeatability of records for cows having at least two production records was found to be 0.32. This is slightly lower than the figure of 0.40 which commonly is accepted as typical of dairy records. Many of the records actually were made under three- and four-time milking conditions, and if the conventional factors for converting three- and four-time records to a two-time basis failed to correct for the conditions prevailing in the University of Nebraska herd, repeatability of the corrected records would tend to be lower than if the correction factors were calculated from the data themselves. This has been brought out in several studies and again recently in a study of the University of Missouri herd by Laben and Herman (9).

Using a repeatability figure of 0.32, deviations from the breed mean of single records were calculated as 32 per cent of the actual deviation, while averages of two, three and four records were calculated as 48 per cent, 59 per cent and 65 per cent of the actual deviations. The mean 305-day, mature-equivalent, two-

time records for the period studied, were 519.8 lb. of butterfat for the Holstein cows and 361.6 lb. of butterfat for the Guernseys. Using the deviation from the breed average has the added advantage that it eliminates from the data any breed differences which existed in the level of production.

The mean squares between and within breeds are given in table 2. Since the F-value for 103 and 1 degrees of freedom is beyond 250 at the 5 per cent level of

TABLE 2

Mean squares between breeds and within breeds of gain in wither height, gain in heart girth and most probable producing ability

Mean square	Degrees of freedom	Wither height gain	Heart girth gain	Most probable producing ability
Between breeds	1	0.00087	0.00013	8.22
Within breeds	103	0.00170	0.00415	1009.38

significance, none of the differences in mean squares is significant. The within-breed correlations between height increase, heart girth increase and most probable producing ability are given in table 3.

Any genetic relationship between heart girth gain and producing ability would have to be determined by the genetic correlation between these two characteristics. A method for determining the genetic correlation between two traits has been given by Hazel (7). This method estimates the genetic correlation from phenotypic correlations of the two traits between daughters and dams. Including both breeds, 47 dam-daughter pairs were available. The 47 daughters

TABLE 3

Within-breed correlations between height increase, heart girth increase and most probable producing ability

	Heart girth gain	Most probable producing ability
	z	y
Wither height gain x	0.153	0.122
Heart girth gain z	.....	0.344

Only r_{xy} is significant. The fiducial limits at the 1% level are 0.55 and 0.10.

were by eight different sires which gives only 38 degrees of freedom to determine the intrasire correlations. The phenotypic correlations are given in table 4.

Only one of these correlations approaches significance at the 5 per cent level. The reciprocal correlations are, however, rather consistent. Heritability estimates may be obtained by doubling the figures in the diagonal from the upper left to the lower right corner. Growth in height thus shows a heritability of 0.48, estimated producing ability 0.60 and chest girth growth – 0.42. The heritability figure for most probable producing ability would be expected to be higher than the heritability figures for single records by $\frac{N}{1+(N-1)\,r}$ where N is the

number of records and r is the repeatability (11). The negative correlation between heart girth growth of daughters and dams may be due entirely to sampling errors, but it indicates that the heritability of heart girth growth rate probably is very low. With a correlation of zero or less for this characteristic, it is not possible to calculate the genetic correlation between growth in heart girth and most probable producing ability. The genetic correlation between height increase and producing ability is $\sqrt{\frac{0.220 \times 0.157}{0.237 \times 0.302}} = 0.69$

DISCUSSION

When the measurement data used for this study originally were collected, only one measurement was taken each time. Thus, there is no direct means of calculating the error of measurements. However, the animals were measured every month, and it therefore is possible to compare the increase from 8 to 10 mo. of age with the increase from 10 to 12 mo. of age. An analysis of variance of

TABLE 4

Intrasire correlations[a] of height increase (x), heart girth increase (z) and most probable producing ability (y) between daughters and dams

Dams	Daughters		
	x'	z'	y'
x	0.24	−0.09	0.22
z	−0.09	−0.21	0.12
y	0.16	−0.04	0.30

[a] These correlations are based on 38 degrees of freedom for which the 5% level of significance is 0.31.

the Holstein records shows that the standard deviations of the differences within animals were 0.0667 cm. for heart girth increase and 0.0412 cm. for increase in height of withers. Since these figures are based on daily gains during a 60-day period, the corresponding standard deviations of differences in measurements taken 60 days apart would be 60 times as large or 4.0 cm. for heart girth increase and 2.5 cm. for height increase.

Touchberry and Lush (15) have given values of the standard deviation due to errors of measurements in dairy cattle. For heifers at 1 yr. of age these values are 0.87 cm. for wither height and 1.08 cm. for heart girth. The standard deviations (due to errors of measurements) of differences between measurements of the same characteristic in the same animal at two different times would be $\sqrt{2}$ times as large as these values or 1.23 cm. for height increase and 1.53 cm. for the heart girth increase. This comparison of the errors of measurement in the two sets of data is made on the assumption that the true rate of growth from 8 to 10 mo. of age is the same as the true rate of growth from 10 to 12 mo. of age. Even if this is unlikely to be exactly true, the comparison indicates that the errors of measurement in the present data are larger than the ones found by Touchberry and Lush (15). Random errors in measurements tend to make the observed correlation smaller than the true correlation (6, p. 364). Since there is no reason to

believe that there are any systematic errors in the measurements, the true correlations may be assumed to be somewhat larger than the observed correlation of 0.34 between increase in heart girth and the most probable producing ability calculated from the lifetime performance records of the animal.

The correlation between the first record of a cow and the average of her subsequent records is $r\sqrt{\frac{N}{1+(N-1)\,r}}$ where r is the repeatability of single records and $(N+1)$ is the total number of records (1). Using the repeatability figure of 0.32 found in the present data, the correlation between the first record and the average of the subsequent two records would be 0.39, which is only slightly higher than the correlation of 0.34 found in the present study between heart girth gain and estimated producing ability. However, since many cows have only one lactation record, the inclusion of heart girth growth rate in any evaluation process to determine the animal's most probable producing potential might add as much information about the individual's producing ability as one more lactation record.

The results give no definite answer to the question of whether measurements of heart girth gain is of any help in evaluating the animal's breeding worth for butterfat production. This relationship would depend upon the heritability of heart girth gain and upon the genetic correlation between gain in heart girth and producing ability.

Touchberry (14) found negative, although statistically insignificant genotypic correlations between size factors and milk and butterfat production. He points out, however, that since the production records were corrected for age, this correction of the production records removes any correlation between size and production which might be accounted for by the correlation of size with age. The evidence in the present study indicates a positive relationship between growth of chest girth and butterfat production. However, the negative daughter-dam correlation (table 4) found in the present data, although insignificant, indicates that the relationship between rate of gain in heart girth and butterfat production is of an environmental nature.

As for growth rate of wither height, the picture is reversed. Here the phenotypic correlation between height increase and butterfat production is low and insignificant, while the genetic correlation is rather large (0.69). This is in harmony with the fact that hormones of the anterior pituitary influence skeletal growth as well as milk production.

Pooling of large amounts of data is necessary to give a more definite answer. The importance of finding an answer is due to the fact that when two traits are considered desirable, any positive relationship between them which is entirely of an environmental nature actually impedes selection progress, as pointed out by Lerner (10, p. 248).

Although the results presented in this paper are statistically insignificant, they do point to a problem of major importance in dairy cattle breeding where the aim is to develop high producing animals and where factors affecting size and growth are considered to be valuable attributes.

SUMMARY AND CONCLUSIONS

A positive correlation of 0.34 was found between rate of growth in heart girth between the ages of 10 to 12 mo. and most probable producing ability. Knowledge of growth rate in heart girth at this age may be a help in evaluating future butterfat production of the individual.

No evidence is found to support the view that this relationship might be of a genetic nature. The results indicate that the relationship may be of an environmental nature. If this is the case, it may have important bearings upon our present procedures of selection in dairy cattle where high production as well as size is considered to be desirable.

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THE FERTILITY OF INTERBREED VS. INTRABREED MATINGS AS DETERMINED FROM ARTIFICIAL BREEDING DATA

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The reasons underlying the practice of crossbreeding dairy cattle may be attributed to a number of factors: (a) Desire to produce smaller calves by first-calf heifers, (b) indifference as to the breed of sire, (c) attempts to increase the butterfat test or milk production, (d) desire to increase the value of progeny for veal purposes, (e) belief by some dairy farmers that conception is enhanced by crossing breeds.

Many dairy farmers believe that interbreed matings improve fertility. The possibility of such matings, especially in commercial herds, has increased markedly as a result of the recent expansion of artificial breeding.

Data by Christian, Dreher, and Casida, and published by Casida (2) suggested the possible influence of breed crossing on breeding failures. However, in a later study including 66 cows, each of which had been bred four or more times previously without apparent conception, Christian *et al.* (3) found no evidence that breed crossing is beneficial in increasing conception rate in repeat-breeder cows.

The object of this investigation was to obtain further information on this problem by comparing the fertility of interbreed matings and intrabreed matings which have occurred in the practice of artificial breeding.

EXPERIMENTAL PROCEDURE

University of Wisconsin artificial breeding records for the period from Oct. 1, 1948, to Oct. 1, 1950, were summarized in making the necessary comparisons. Of the 44,056 first-service inseminations made during this 2-yr. period, a total of 1,576 services by 22 Holstein-Friesian bulls, and 1,116 services by 14 Guernsey bulls was studied. Fertility was measured by 60- to 90-day non-returns and was based on first-services to Holstein-Friesian and Guernsey cows in Dane and Sauk County herds. The interbreed inseminations were compared with intrabreed inseminations made on the same day, by the same technician and with semen from the same bull. No attempt was made to balance the frequency of the within- and between-breed matings of each bull.

As in most artificial breeding data, the breed of the cows was established from reports submitted by the field technicians. Only cows reported as Holstein-Friesian or Guernsey were included in the study. Three per cent of the 1,576 first-service Holstein-Friesian cows and three per cent of the 1,116 first-service Guernsey cows employed in this trial were registered animals. Only one of

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these registered cows was mated to a bull of a different breed. Of the total first-service inseminations made by the University stud during the 2-yr. period of the study, 4.4 per cent were to registered cows.

The results were tabulated for individual bulls and summarized for both breeds of the 36 bulls. The data were analyzed by Chi-square, using techniques described by Norton (7) and Snedecor (8).

RESULTS AND DISCUSSION

Results from matings involving Holstein-Friesian and Guernsey bulls are summarized in table 1. The intrabreed fertility of all Holstein-Friesian bulls was 67.4 per cent, while the intrabreed fertility of the Guernsey bulls was 65.7 per cent. This conception rate was slightly higher than the total non-returns for the University stud during the period studied; however, the difference was negligible when individual bull frequencies were considered. The Chi-square values for interbreed vs. intrabreed matings involving the 22 Holstein-Friesian bulls ($P = 0.25$) and the 14 Guernsey bulls ($P = 0.95$) were not statistically significant.

TABLE 1

Breeding efficiency of interbreed and intrabreed inseminations involving semen from Holstein-Friesian and Guernsey bulls

Breed of bulls	No. bulls	Guernsey cows		Holstein-Friesian cows		Diff. % Non-ret.	Chi[a] square
		No. serv.	% Non-ret.	No. serv.	% Non-ret.		
Unadjusted:							
Holstein	22	421	70.5	1,155	67.4	3.1	1.37
Guernsey	14	699	65.7	417	65.5	0.2	0.00452
Adjusted:							
Holstein	22	389	68.1	1,155	67.4	0.7	0.0610
Guernsey	14	688	65.0	373	61.4	3.6	1.45

[a] Chi-square value of 3.84 required for $P = 0.05$.

Prior to the analysis it was observed that in 49 services, errors were made in breed identification. These errors were found as a result of the technic employed in checking first-services for repeats. Since this technic made it possible to discover these errors only on repeat services, it appeared necessary to make an equivalent adjustment on the first-services that were not repeats. This was done by obtaining the frequency of these false breed identifications in services classified by the four types of matings. The adjustments, based on the percentage errors among repeat services within each group, then were made on the first-service animals within the same group that were not repeats. The adjusted data are presented in table 1. The Chi-square values for interbreed vs. intrabreed matings ($P = 0.81$ and $P = 0.24$) were not statistically significant.

The results obtained in this study indicate that no increased fertility was obtained from interbreed vs. intrabreed inseminations made on the same day, by the same technician and with semen from the same bull. The interaction Chi-

square (7) between bulls within each breed was not statistically significant, indicating that any observed variation among bulls in regard to mating comparisons should not be considered real.

The breed effect of sires used in artificial breeding establishments has not been thoroughly characterized in the literature. However, Lewis (5) reported the breeding efficiency of Guernseys as being significantly lower (6.3 per cent) than that of Holstein-Friesians in commercial artificial insemination. Similar results were reported by Mercier and Salisbury (6) involving the same breeds in artificial insemination in New York.

In the present study, intrabreed inseminations involving Holstein-Friesian bulls were 2.4 per cent ($P > 0.05$) higher than Guernsey bulls (adjusted data, table 1) when analyzed by Chi-square. When all matings were considered, the fertility of Holstein-Friesian bulls was 3.8 per cent ($P < 0.05$) higher than Guernsey bulls. This breed difference corresponds closely to the statistically significant difference of 3.6 per cent obtained from the 44,056 first-service inseminations by the University stud during the period studied (1). Since no difference was found between intrabreed and interbreed matings by the same bull, it appears that breed of bulls has greater effect on fertility than breed of cows.

SUMMARY

This investigation included 2,692 inseminations by 22 Holstein-Friesian and 14 Guernsey sires. There was no evidence from these data that any real difference in fertility exists between interbreed and intrabreed inseminations made on the same day, by the same technician and with semen from the same bull. Some indication of breed of bull effect on fertility was suggested.

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ABSTRACTS OF LITERATURE

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BUTTER

O. F. HUNZIKER, SECTION EDITOR

569. Advantages of continuous buttermaking system in a southern plant. T. B. HARRISON, Univ. of Tenn., Knoxville. Sou. Dairy Prod. J., **51,** 5: 100, 101, 104, 105. May, 1952.

Employees of the Sugar Creek Creamery, Knoxville, Tenn., claimed the following advantages of the Gold'n Flow continuous buttermaking stystem: Improved quality, including body and texture; better keeping quality; more sanitary equipment because of all stainless steel construction and ability to completely disassemble; elimination of leakiness; thorough distribution of salt; reduction of fat losses by 50%; lower labor requirement; elimination of 2/3 the steam and refrigeration necessary for pasteurizing and cooling by the old method and increased efficiency in record keeping. F. W. Bennett

570. Device for handling butter and like materials. A. H. RISHOI, C. F. WEINRICH and L. BURRELL (assignors to Cherry-Burrell Corp.). U. S. Patent 2,608,759. 5 claims. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 79. 1952.

A metal churn and butterworker has the inner surface roughened in such a manner that when it is wet, the butter will not adhere to it. A chrome finish, containing at least 18% Cr., is applied to rust-proof the churn and parts.
R. Whitaker

571. The catalase test as a means to detect contamination with mirco-organisms in butter. TH. E. GALESLOOT. Netherlands Milk & Dairy J., **3,** 2: 111–116. 1949.

A catalase test for butter is described but the author concluded the test is not always reliable for detecting contamination of butter. Some butters highly contaminated with yeasts or bacteria gave a catalase number equal to butter that was not contaminated. With some yeast the low pH of the butter decreased the catalase number.
W. W. Overcast

572. Routine determination of copper, iron and manganese in butter. P. C. DEN HERDER and B. M. KROL. Netherlands Milk & Dairy J., **4,** 1: 42–53. 1950.

Methods are described for determination of Cu, Fe and Mn in butter, which the authors feel are suitable for routine work and are sufficiently accurate for practical purposes. Determinations are carried out on the serum after it has been separated from the butterfat. W. W. Overcast

573. The physical structure of the birefringent layer of the fat globules in buttter. N. KING. Netherlands Milk & Dairy J., **4,** 1: 30–41. 1950.

The birefringent layer of the fat globule in butter is a spherical layer-crystal built up of radially orientated rod-like molecules of higher melting butterfat fractions. Sometimes the layer-crystal is segregated into small needle crystals orientated tangentially to the globule surface. In cooled cream the birefringent layer is present on only a few of the fat globules. However, if an optimum pressure is exerted on the globules, the optically active peripheral layer is formed.
W. W. Overcast

574. Electric coolers for maintenance of cream quality on the farm. E. M. SHERWOOD and A. W. FARRALL, Mich. State College, East Lansing. Sou. Dairy Prod. J., **50,** 6: 98–100. Dec., 1951.

A low cost electrically refrigerated dry-box type of chamber for cooling and storing cream has been built at Michigan State College. Comparisons were made from Aug.–Sept., 1950, on farms near Evansville, Ind., between cream held about 4 d. in the cooler before delivery and duplicate portions held without refrigeration. An average of 24.9 lb. were cooled in cooler *A,* which had a capacity of one 10-gal. can. The current consumption was 4.6 kwh/wk. The average acidity of the cream was 0.206% as compared with 0.428% in the unrefrigerated cream. An average of 60.2 lb. was cooled in cooler *B,* which had a capacity of two 10-gal. cans and a fan to circulate air around the cans. The current consumption was 20.3 kwh/wk. The average acidity of the cooled cream was 0.265% and that of the uncooled cream was 0.572%.
F. W. Bennett

575. The decline of butter . . . what can be done about it. W. GRELL, Armour & Co., Chicago. Sou. Dairy Prod. J., **50,** 6: 111–114. Dec., 1951.

A 50% decline in butter consumption has occurred during the past 10 yr. as a result of a limited supply being available and of regulations during the war. Two primary problems in restoring a higher rate of consumption confront the industry: (a) Make butter of outstanding keeping

quality and (b) maintain its fine flavor during processing and distribution. Holding printed samples in the laboratory at 70° F. for 7 d. and re-examining them, may fairly accurately measure the keeping quality. Screw-type printers are commonly detrimental to keeping quality. Contamination of the butter with Cu, Fe, Co, Ni and other metals from equipment, wrappers or other sources is important in catalyzing fat changes, particularly oxidation. F. W. Bennett

CHEESE

A. C. DAHLBERG, SECTION EDITOR

576. The influence of the temperature on the ripening of Edam cheese. C. W. RAADSVELD and H. MULDER. Netherlands Milk & Dairy J., **3,** 2: 117–141. 1949.

Several series of Edam cheese were made from raw milk and changes in chemical composition and physical properties were noted on cheese ripened at 9 and 17° C. The protein breakdown at 17° C. in 200 d. of ripening was about comparable to 500 d. at 9° C. However, the acidity of the fat was much higher at the elevated temperature. The cheese ripened at the higher temperature was described as having a flavor of the volatile fatty acids. The authors concluded that a short ripening period at a higher temperature, in practice, generally did not produce a cheese equal to the prolonged ripening at a lower temperature. W. W. Overcast

577. The influence of pH on the ripening of Edam cheese. C. W. RAADSVELD and H. MULDER. Netherlands Milk and Dairy J., **3,** 4: 222–230. 1949.

Two lots of Edam cheese were made from raw milk with different pH values. They were ripened for approximately 500 d. at 9 and 17° C. In general, the results indicate a greater breakdown of protein occurred in cheese with the higher pH. Fat acidity always was higher in cheese with lower pH. Volatile fatty acids were felt to be the contributors of flavor to the acid cheese, while the protein breakdown products were responsible for bitter and sweet flavors of cheese with higher pH levels. W. W. Overcast

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

578. The application of nomograms to the analysis of dried milk. TH. BROUWER. Netherlands Milk & Dairy J., **4,** 2: 81–95. 1950.

Five nomograms are presented and their construction and use are explained.
W. W. Overcast

579. Method of preserving spray-dried food products. P. F. SHARP and A. J. WASSON (assignors to Golden State Co., Ltd.). U. S. Patent Reissue 23,548. 10 claims. Sept. 9, 1952. Official Gaz. U. S. Pat. Office, **662,** 2: 363. 1952.

U. S. Patent 2,541,441 (J. Dairy Sci. Abs. #191, 1951) has been reissued. It is concerned with gas packing of dried milk to reduce oxidation to a minimum. R. Whitaker

580. Apparatus for treatment of liquids in containers. J. O. FOWLER (assignor to Fowlers Dairy Development, Ltd.). U. S. Patent 2,607,284. 2 claims. Aug. 19, 1952. Official Gaz. U. S. Pat. Office, **661,** 3: 691. 1952.

Liquid food products, such as milk, are sterilized in bottles by immersion in hot water in a sterilizer. To prevent the sterilizing water from entering the bottles when not tightly sealed, especially when being cooled after sterilizing, the bottles are placed in racks, and over each case is placed an inverted tray which provides an air lock. The trays are shaped for easy stacking when not in use. R. Whitaker

581. Product sterilizing apparatus and method. W. MCK. MARTIN (assignor to James Dole Engineering Co.). U. S. Patent 2,607,698. 9 claims. Aug. 19, 1952. Official Gaz. U. S. Pat. Office, **661,** 3: 807. 1952.

A continuous sterilizer for foods in cans, in which the cans are conveyed through a heating and cooling zone by a bar conveyor is described. The contents are agitated by rolling the cans in alternating directions by frequently changing the direction of the belt on which the cans roll. Super-heated steam is employed as the heating medium, so that temperatures above 212° F. may be obtained at atmospheric pressure.
R. Whitaker

582. Puncturing dispenser for canned liquids. H. M. EVANS. U. S. Patent 2,609,121. 1 claim. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 181. 1952.

A can puncturing and holding device for dispensing evaporated milk from cans is described.
R. Whitaker

583. Food supplement for animals and poultry. R. W. HAYES. U. S. Patent 2,608,483. 9 claims. Aug. 26, 1952. Official Gaz. U. S. Pat. Office, **661,** 4: 1094. 1952.

A semi-solid or paste-like feed consists of fermented whey concentrated with 5–25% of buttermilk or skimmilk and mixed with 10–50% of soluble fish materials and 1–5% brewer's yeast.
R. Whitaker

584. Correlation between bacterial infection and sweet curd in evaporated milk. A. VAN KREVELD. Netherlands Milk & Dairy J., **4,** 2: 115–120. 1950.

The authors felt that the sweet curd sometimes formed in evaporated milk was not due to bacterial action but could be due to some physico-chemical mechanism. W. W. Overcast

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

585. Zur Kenntnis und Systematik der in der Milch vorkommenden Mycobakterien (Information concerning the systematic classification of mycobacteria found in milk). English summary. A. MEYN and H. STÖFFLER. Milchwissenschaft, **6,** 1: 15–18. Jan., 1951.

Of 66 samples of raw milk clarifier slime from various milks, 16 contained acid-fast bacteria of the mycobacteria type. Further classification according to Bergey's Manual revealed that 4 of the above 16 samples contained *Mycobacterium tuberculosis,* var. *bovis,* 8 *M. lacticola* and 4 *M. phlei.* I. Peters

586. Zur Gäranlage und Reifung der Käsereimilch im Zusammenhang mit der Käsereitauglichkeit von Silage milch (Concerning the fermentation and ripening of cheese milk in connection with the suitability of silage milk for cheese). English summary. J. Mosle. Milchwissenschaft, **6,** 1: 21–24. Jan., 1951.

Butyric acid fermentation in milk could be prevented by adding 0.01–0.1% NH_4NO_3 to heated milk prior to inoculation with dirt and silage juice containing butyric acid bacteria. In the presence of metals, *e.g.,* Al, Zn, Fe, butyric acid fermentation took place even though NH_4NO_3 was added.

Miscroscopic examinations of cultures failing to produce butyric acid in the presence of NH_4NO_3 showed a predominance of cocci. These cocci grew well at 37° C., were heat resistant up to 100° C., produced acid but did not reduce nitrates. I. Peters

587. The influence of some oxidizing agents on butyric acid fermentation in cheese. E. A. Vos. Netherlands Milk & Dairy J., **3,** 4: 231–244. 1949.

Several compounds that were capable of retarding the butyric acid fermentation of *Cl. tyrobutyricum* in culture media were tried in Edam cheese. None of the compounds was effective in the cheese, as they were not found to be present when the butyric acid fermentation began. W. W. Overcast

588. An early gas defect in cheese caused by betacoccus (Leuconostoc) species. Th. E. Galesloot. Netherlands Milk & Dairy J., **4,** 4: 274–288. 1950.

Certain lots of cheese made from milk with added $KClO_3$ and practically free of coli-aerogenes bacteria and lactose-fermenting yeast showed an early gas defect. From these cheese samples, *Betacoccus arabinosaceus* (*Leuconostoc mesenteroides*) and *Betacoccus bovis* (*Leuconostoc dextranicum*) were isolated. Cultures of these organisms were capable of producing early gas in experimental cheese. However, when the lactic acid-producing organisms were very active the defect was absent or slight. The normally occuring aroma-producing organisms present in a cheese culture were not felt to be serious. W. W. Overcast

589. Some factors which contribute to the psychrophilic bacterial count in market milk. F. A. Rogick and L. H. Burgwald, The Ohio State Univ., Columbus. J. Milk & Food Technol., **15:** 181–185. July–Aug., 1952.

The total bacterial counts on fresh raw milk obtained from several sources showed mesophilic counts were 3 times greater than psychrophilic organisms. Milk pasteurized by holder or HTST showed no psychrophilic bacteria. However, when samples were held in cold storage for 7 d., the psychrophilic counts were much higher than the mesophilic counts. Psychrophilic organisms were found in the 1st bottled milk but not in the last. Morphological and physiological studies indicated that the organisms were largely cocci or non-spore-forming bacilli and were inert or acid producing, facultative rather than true psychrophiles. H. H. Weiser

590. Über akute Probleme der Molkereibakteriologie (Concerning acute problems in dairy bacteriology). English summary. K. J. Demeter. Milchwissenschaft, **6,** 1: 18–20; **2:** 48–51. Jan., Feb., 1951.

The author discusses problems of bacteriological nature concerning (a) various methods of milk pasteurization or its equivalent; (b) starter difficulties, including bacteriophage and aroma production; (c) keeping quality of butter; (d) influence of feeding of silage upon suitability of milk for cheese making, with special reference to butyric acid bacteria; (e) influence of pasteurization upon cheese milk and the aroma of ripened cheese. 76 selected references. I. Peters

591. Retention of milk and milk product films on glass slides during staining, defatting and rinsing for microscopic examination. N. A. Milone, Univ. of Mich., Ann Arbor. J. Milk & Food Technol., **15:** 195. July–Aug., 1952.

Clean glass slides are important in retaining a uniform distribution of milk on slide. A coating of Bon Ami on the glass slide after it has been washed and dried and allowed to remain until used has been successful. H. H. Weiser

592. Resazurin test for milk. N. S. Golding (assignor to State College of Washington Research Foundation). U. S. Patent 2,609,275. 1 claim. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 225. 1952.

To facilitate conducting the resazurin test for milk quality, the required amount of resazurin is dried in sterilized capped vials. The vials, ready for the sample of milk, are stored until used. R. Whitaker

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

593. The ultracentrifugation of calcium caseinate sols. Maria G. Ter Horst. Netherlands Milk & Dairy J., **4,** 4: 246–260. 1950.

In an attempt to study adsorption of ions by casein, the Ca caseinate was fractionated in the ultracentrifuge. These various fractions differed in Ca-combining capacity, isoelectric point, PO_4 content and lysine and made it difficult to study ion adsorption. However, data presented show casein can combine with considerable quantities of Na and Cl ions even in the presence of a large excess of Ca. W. W. Overcast

594. Probleme der Eiweiss—Molekülstruktur (Problems related to the structure of protein molecules). English summary. P. Jordan.

Milchwissenschaft, **6,** 1: 12–14. Jan., 1951.

Astbury's theory of chain structure has been displaced by Barnal's assumption of a ring structure, based on Miss Crowfoot's work with X-rays.

The author believes that protein molecules containing 1 or more rings grow by autocatalysis up to a certain size, whereupon they polymerize (join) and form larger molecules. This reasoning is in agreement with Svedberg's discoveries and is expressed by the emperical formula 2^n3^m.

I. Peters

595. Lactalbumin purification. J. D. Ingle and R. T. Stahl (assignors to Swift & Co.). U. S. Patent 2,607,766. 3 claims. Aug., 19, 1952. Official Gaz. U. S. Pat. Office, **661,** 3: 821. 1952.

Ca and Mn impurities are removed from lactalbumin by washing with an acidified water at pH 2.0–5.0 at a temp. of 100–170° F.

R. Whitaker

596. Chemisch-physikalische Gleichgewichtssysteme in der Milch (Chemical-physical equilibrium systems in milk). English summary. O. Ballarin. Milchwissenschaft, **6,** 1: 6–10; **2:** 38–42. Jan., Feb., 1951.

A theoretical discussion is based on established facts and various theories dealing with the numerous co-existing equilibrium systems in milk. This is well presented and includes appropriate schematic illustrations. I. Peters

597. Beseitigung der Gefahr von Ätherexplosionen beim Abäthern auf Heizplatten (Removing the danger of ether explosions during evaporation on hot plates). English summary. W. Mohr. Milchwissenschaft, **6,** 1: 24–25. Jan., 1951.

Description of an apparatus is presented, including diagram, which permits the safe evaporation of a large number of Stoldt or Roese-Gottlieb fat determination flasks. I. Peters

598. Investigation of the fatty acids of summer butter. II. A. Kentie. Netherlands Milk & Dairy J., **3,** 3: 182–199. 1949.

The methyl esters of fatty acids of summer butter were fractionated and converted into glyceride esters. These various fractions were fed to rats. A fraction with a molecular weight of 292.5 and an iodine value of 70.1 apparently contained a growth-promoting factor. The investigator felt that probably vaccenic acid was responsible for additional growth of rats. Partially purified fractions of vaccenic acid did give additional growth, but almost pure vaccenic acid showed no growth-promoting properties.

W. W. Overcast

599. Separation and estimation of saturated C_2-C_7 fatty acids by paper partition chromatography. R. L. Reid and M. Lederer, McMaster Animal Health Lab., Sydney, Australia. Biochem. J., **50,** 1: 60–67. 1951.

A method is described for estimating the individual fatty acids by paper partition chromatography. It is claimed that an accuracy of ±2–5% is possible. A. O. Call

600. Process of protecting fatty and phosphorous lipoidic food products, particularly milk and dairy products, margarine and the like, against getting unpleasant taste or flavor. C. G. E. Sjostrom and A. V. Larsson. U. S. Patent 2,609,297. 9 claims. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 231. 1952.

Rancid flavors are avoided by treating fat-containing dairy products with a protecting agent made by treating, over a period of time, a reducing sugar, in the absence of air, with an alkali at 80–95° C., within the pH range of 9–11.5.

R. Whitaker

601. The detection and determination of neutralizing agents and/or lactic acid in liquid milk and dried milk. I. The detection of neutralizing agents. C. I. Kruisheer. Netherlands Milk & Dairy J., **4,** 1: 22–29. 1950.

Three methods were investigated for accuracy in determining neutralizing agents present in milk. These were Kolthoff's method for alkalinity of ash, the Barber and Kolthoff method for determination of sodium and the method of Tillmans and Huckenbach for buffering power of the serum. None of these methods was sufficiently accurate for quality control. W. W. Overcast

602. The detection and determination of neutralizing agents and/or lactic acid in liquid milk and dried milk. II. The colorimetric determination of lactate according to Heinemann. P. Tiersma and J. T. N. Venekamp. Netherlands Milk & Dairy J., **4,** 3: 158–164. 1950.

A detailed method is presented for determination of lactates in liquid or dried milk. However, it was felt that the method was not suitable for routine dairy laboratories. W. W. Overcast

603. The detection and determination of neutralizing agents and/or lactic acid in liquid milk and dried milk. III. A colorimetric routine method for the estimation of lactic acid (lactate) in milk and dried milk. J. T. N. Venekamp and C. I. Kruisheer. Netherlands Milk & Dairy J., **4,** 3: 165–174. 1950.

The lactate is determined by addition of dilute $FeCl_3$ to a serum obtained by adding Fe_2O_3 sol to the milk. The intensity of yellow color is measured photometrically and lactic acid or lactate content is derived from a standard curve. By comparing the lactic acid content with milk pH, the amount of added neutralizer can be determined. W. W. Overcast

604. On the titration curve of skimmilk. H. J. C. Tendeloo, A. E. Mans and Miss G. De-Hoogh. Netherlands Milk & Dairy J., **3,** 3: 178–181. 1949.

A theoretical system is presented for the titration curve of skimmilk, based on the assumption that skimmilk may be considered as a system of poly-acids with gradually changing dissociation constants. W. W. Overcast

605. The composition of Friesian milk. L. C. Janse. Netherlands Milk & Dairy J., **4,** 1: 1–9. 1950.

A comparison of the composition of Friesian

milk of 1927–28 is made with that of 1948–49. The data show increases of 0.54% in fat and 0.38% in the solids-not-fat over the 21-yr. period.
W. W. Overcast

606. Die Bedeutung der Farbenfotografie für die Darstellung milchwissenschaftlicher Untersuchungen (The importance of color photography in the presentation of dairy research). English summary. A. KÜHNEL. Milchwissenschaft, **6,** 1: 25–28. Jan., 1951.

The author points out the value of color photography in dairy research. He explains the theory of color photography and suggests methods for obtaining best results. Three illustrations of color photography are included. I. Peters

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. G. THOMSEN, SECTION EDITOR

607. Dating of milk. H. POLIKOFF, New York. Milk Dealer, **41,** 10: 126–133. July, 1952.

It is the requirement of many jurisdictions that milk bottle caps or the containers in which milk is sold shall expressly show the day of the week upon which the milk was pasteurized or delivered by the dealers. Only 7 of the 20 largest cities in the U. S. require dating; these 7 are among the 10 largest. Only 13 of the 44 major cities throughout the U. S. require dating. Locations of these cities and purpose of dating are discussed. The author concludes that dating is a burden rather than a benefit to the public interest because: (a) Dating is an antiquated requirement which, while once desirable, is no longer so. (b) Dating as an enforcement technique with respect to a time limit for the sale of milk is unreasonable, because it prevents the sale of milk while it is still lawful to sell it. (c) Dating misleads the public since quality age is more important than the calendar age of milk, and these are two different things. Dates do not make or keep milk safe. Supervision of the milk supply must rest with public officials. (d) Dating is costly and wasteful, and, in the last analysis, consumers must pay the bill. (e) Dating reduces consumption and tends toward poorer quality of milk; it creates more evils than it can remedy.
C. J. Babcock

608. What's ahead for butterfat and solids-not-fat. R. ALBERTS, American Butter Inst., Chicago, Ill. Ice Cream Rev., **36,** 1: 66–68. Aug., 1952; Milk Dealer, **41,** 11: 82–85. Aug., 1952.

Wholesale substitution of vegetable fat for butterfat inevitably will lead to reduction in value of butterfat and an increase in value of milk solids-not-fat. If the price of milk solids-not-fat, however, becomes too high, there may be a trend towards substitution of cheaper solids for solids-not-fat. To combat such a situation the entire dairy industry must unite to establish the fact that, regardless of price, there is no substitute for milk solids-not-fat in human nutrition. If the day should come when milk solids-not-fat are replaced with substitute products, the dairy industry will be doomed to oblivion. W. J. Caulfield

609. Threat of imitations. H. H. SOMMER, Univ. of Wis., Madison. Ice Cream Rev., **35,** 12: 117–119. July, 1952.

Vegetable fats are making inroads on the use of butterfat by American consumers. This is true of butter as well as other products such as filled evaporated milk, frozen desserts and whipping cream. If this trend continues unchecked, the price of butterfat will, of necessity, decline to nearly the same price as other high quality food fats. To offset a decline in the price of butterfat there must be a compensating increase in the price of milk-solids-not-fat to sustain milk production. This shift in our pricing system must be made gradually to avoid disastrous consequences.

The dairy industry might slow down this trend by gathering authoritative evidence to show the extent to which butterfat is being substituted in dairy products with other fats either legally or illegally. Such evidence then could be presented to legislative bodies for action. Authoritative evidence regarding the illegal substitution of butterfat also would prove valuable in motivating enforcement officials to become more active in checking such abuses. Such actions can only serve to delay the trend.

Plans must be formulated whereby the milk-solids-not-fat will be sold at a much higher price level than at present. In the immediate future, however, butter must continue to bear the major share of milk prices. To make this possible it is suggested that good butter should not have to compete in the market with low quality butter produced from farm-separated cream, improperly cared for. It also is suggested that price supports should not be applied so as to support the continuance of marginal dairying.

The need for a simple practical method for testing milk for solids-not-fat is emphasized.
W. J. Caulfield

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

610. Flavor of milk as affected by grazing cows on ladino clover. J. J. SHEURING, R. A. MARDEN, S. H. MORRISON and J. F. DEAL, Univ. of Ga., Athens. Sou. Dairy Prod. J., **51,** 5: 34, 35, 38, 39, 46–48, 62, 64. May, 1952.

Cows were grazed on a winter pasture of ladino clover, fescue and orchard grass. Flavor scores showed no clover flavor in the milking following regular grazing on the mixture for 2, 4 and 10 hr. immediately after milking. F. W. Bennett

611. The digestibility of carotene by the cow and the goat as affected by thyroxine and thiouracil. R. CHANDA, H. M. CLAPHAM, MARY L. MCNAUGHT and E. C. OWEN, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **50,** 5: 95–99. 1951.

The rate of carotene conversion to vitamin A by the cow and goat appears to depend upon the thyroid activity of the animal. When the carotene in the diet was reduced, fecal carotene diminished. It increased again, however, when

the carotene level in the diet was increased. When thyroxine was injected subcutaneously, reappearance of fecal carotene was much later, but when thiouracil was injected, reappearance was much earlier. The digestibility of carotene in both cows and goats was increased by thyroxine injection and decreased by thiouracil.

A. O. Call

612. Studies on the digestion of a ruminant. I. The biological characteristics of the aerobic bacteria in the rumen. M. UMEZU, T. TUDA, M. KATUNA, S. OMORI and S. MINAGAKI. Tohoku J. Agr. Research, **2,** 1: 63–71. 1951.

Rumen contents of 4 yearling bovine animals were sampled by stomach catheter before morning feeding and cultured aerobically. One hundred two strains of pure culture were isolated on nutrient agar plates and classified into 4 groups based on their biological characteristics. Group A included 65% of the isolated strains, and cultures grew both aerobically and anaerobically. These cultures decomposed arabinose, glucose, lactose and mannit, producing acid and gas, but did not decompose starch. Group B, including 12% of the cultures, was similar to group A in all respects tested, except that the bacteria of this group produced indol from peptone. Cells from groups A and B were gram negative rods.

Cells of groups C and D were gram positive rods which grew anaerobically less than aerobically. Group C cells broke down arabinose, glucose and mannit to acid and decomposed starch, whereas cells of group D decomposed starch and mannit but not arabinose, glucose or lactose. Groups C and D comprised 6 and 18%, respectively, of the 102 cultures studied.

G. E. Stoddard

613. Studies on the digestion of a ruminant. II. Cellulolytic power in the rumen of the goat, in vivo and in vitro. M. UMEZU, T. TUDA, M. KATUNA, S. OMORI and S. MINAGAKI. Tohoku J. Agr. Research, **2,** 1: 73–81. 1951.

Cellulose digestion was studied in a kid using a silk bag introduced orally into the rumen. The bag was withdrawn at intervals of 5, 10, 15, 20 and 24 hr. after introduction and % cellulose (filter paper) digestion calculated. Only about 10% of the cellulose was digested in 5 hr., but in 15 hr., 30–50% of the cellulose had been broken down. By 20–24 hr. cellulose digestion reached a maximum of 60–70%.

Cellulose was added to a flask inoculated with rumen contents from a mature goat and incubated for 10 d. at 37° C. Periodically samples were taken and heat-sterilized to stop any further reaction. These samples then were analyzed for cellulose, glucose and cellobiose. The 120-mg. cellulose sample was reduced by 30 mg. on the 3rd day and an increasingly greater quantity each day until the end of the 10-d. period, at which time 105 mg. had been broken down. During this same period, cellobiose production increased from 1.0 mg. on the 4th day to 10.7 mg. on the 10th day. Glucose was present at a level of 1.2 mg. during each of the 1st 5 d., increasing by the 7th day to 2.7 mg. and decreasing to 0 on the 9th and 10th days.

The authors discussed the possible independent and interdependent natures of aerobic and anaerobic rumen microorganisms in cellulose digestion.

G. E. Stoddard

GENETICS AND BREEDING

N. L. VANDEMARK, SECTION EDITOR

614. Relation between type and butterfat production of Jersey cows in Canada. J. C. RENNIE, Iowa State College, Ames. Sci Agr., **31,** 12: 553–558. 1951.

The butterfat records, converted to a 305-day twice-a-day basis, of 3,328 cows which had been officially classified into 5 type classes ranging from "Excellent" to "Fair" were the basis for this statistical study. The data were analysed to determine (a) the phenotypic correlation between type and fat production in the same cow, (b) heritability of fat production, (c) heritability of type and (d) genetic correlation between type and fat production.

The intra-sire phenotypic correlations between type and a single butterfat record were +0.13, +0.13 and +0.21, for all cows, for daughters and for dams, respectively. These values are all highly significant. The over-all correlation was +0.245. The intra-sire regression of butterfat production on type rating was 11.6 lb., indicating that average production is expected to increase 11.6 lb. for each increaes in type rating

Heritability of fat production was estimated to be 36% transmitted from parents to offspring and type 16%, although both estimates contain large sampling errors.

O. R. Irvine

615. Heritability of udder proportion in dairy cows. I. JOHANSSON and N. KORKMAN, Inst. of Animal Breeding, Uppsala, Sweden. Hereditas, **38:** 131–151. 1952.

By use of a specially constructed milking machine to measure the yield from each quarter separately, data on udder symmetry was obtained from 22 herds representing 3 breeds and from 12 sets of monozygotic twins. The herds were selected to represent different phases of improvement in each breed. Indices were calculated to portray the relationship of front to rear and left to right both with respect to the entire udder and to the front and rear halves.

The left half of the gland was found to produce the same amount of milk as the right side. The variation found was concluded to be wholly non-genetic, with major emphasis on sub-clinical mastitis and coincidental development of the glands. The front quarters produced 41% of the total yield. The heritability of the relative yields between front and rear was estimated to be around 0.75, suggesting that selection for front-to-rear udder symmetry should prove successful. The close agreement between repeatability and estimates of heritability suggested that the inherited influence is caused by genes with additive effects only and that dominant and

epistatic genes played no role. Possibly the inheritance is fairly simple. L. O. Gilmore

616. "Dwarfism" in Aberdeen-Angus cattle. M. L. Baker, C. T. Blunn and M. Plum, Nebraska Agr. Expt. Sta., Lincoln. J. Heredity, **42,** 3: 141–143. 1951.

Occurrence of dwarfism in Aberdeen-Angus is reported for the 1st time. Dwarfs in this area differ from Shorthorns in not always being distinguishable at birth and in exhibiting higher condition early in life. The data analyzed by the method of Li support earlier observations that the condition is due to a single autosomal recessive gene. Statistical support is not given to the opinion that the frequency of the responsible gene is increasing in the breed studied.
L. O. Gilmore

617. Observations on midgets in beef cattle. C. E. Lindley, Washington Agr. Expt. Sta., Pullman. J. Heredity, **42,** 5: 273–275. 1951.

This report contains further observations on the occurrence of dwarfism in Aberdeen-Angus and Hereford cattle (see Abs. 85, J. Dairy Sci., **34:** 15. 1951.). Measurements on 1 dwarf bull and 1 dwarf heifer were compared to normal cattle with respect to weight, height at withers and hips, length of body, cannon bone, eye to muzzle and poll to muzzle and width of head. In all measurements except head width the midgets were below normal. Histological sections showed large cystic spaces in the hypophysis partially filled with reddish hyalin material. The acini of the thyroid were widely dilated and also filled with colloid material. The monofactorial autosomal incomplete recessive explanation for the behavior of the causative gene did not fit 1 case in which a 1,365-lb. cow gave birth to a midget (dwarf) calf.
L. O. Gilmore

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

618. Keeping cows fly-free. E. M. Searls, Natl. Dairy Prod. Co., Inc., New York. Milk Dealer, **41,** 10: 44–45, 52–54. July, 1952.

Information is given on selection of sprayers and insecticides to control flies on cattle and in the stable. An insecticide atomized evenly into the atmosphere of the stable at the rate of about 2 oz./1,000 ft.^{3} will kill or drive nearly all the flies from the stable. By using the aerosol bomb at the rate of about 2 sec. of exposure per 1,000 ft.^{3} of air, the dairyman can kill practically every living insect in the stable. To protect animals in the field, some residue of the insecticide must remain on the cows. About 1 pt. of water dilution spray or 1.5–2 oz. of oil base spray are necessary on the body of the animal for residual repellency. An insecticide which is quite stable and will persist over a period of several hr. must be applied directly to the animals in such a way that they will have a uniform covering. Only methoxychlor and pyrenone, a mixture of pyrethrum and piperonyl butoxide, should be used directly on the animals for general insecticidal purposes. C. J. Babcock

619. Swedish "rubber cowshed" reduces milk production costs. R. Rausing, Simontorps Sateri, Blentarp, Sweden. Milk Dealer, **41,** 11: 48–49, 96–97. Aug., 1952.

The Simontorp rubber cowshed is described. It is designed to save labor in the cowshed and to establish a satisfactory manure economy. The floor is constructed by vulcanizing rubber on sheet metal which then is fixed down by screws to iron set in concrete. The rubber strips are vulcanized together, leaving no seams. This floor can be completely sterilized if necessary. The roof consists of siporex blocks plus siporex slabs with ventilation ducts and over this double layers of maintenance-free roofing. The roof is ventilated under this roofing material. The walls are especially simple and completely maintenance-free. There is no window in the walls. In the roof are lanterns of varied construction and the whole is supported by an iron framework. The overhead lighting thus is evenly distributed and the cowshed well lighted. Air intake is arranged partly through vents and partly by means of a fan; outtake is through air ducts.
C. J. Babcock

620. Milking system and apparatus therefor. G. R. Duncan. U. S. Patent Reissue 23,543. 26 claims. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 57. 1952.

U. S. Patent 2,498,401 (J. Dairy Sci. Abs. #370, 1950.) has been revised to include a means for maintaining the cans in an upright position, while cooling the milk in a refrigerated cabinet.
R. Whitaker

621. Cold wall milk cooling tank. H. C. Goslee, Conn. State Dept. Farm and Mkts., Hartford. Milk Dealer, **41,** 11: 74–77. Aug., 1952.

The items of major interest in bulk milk cooling on the farm are discussed under headings of laws and ordinances, equipment standards, operational methods, product standards, consumer protection and producer protection. Continued approval of the farm tanks will in no small measure be dependent upon the satisfactory performance of the project from cow to plant. Continuous transportation of the producer composite samples is open to criticism. The present method of transferring milk from farm tank to truck tank and then from truck tank to storage tank at the plant through a positive pump needs study. An early agreement among the weights and measures officials concerning the basic points in tank calibration is needed. C. J. Babcock

622. Milk cooler. O. H. Wennlund. U. S. Patent 2,610,035. 1 claim. Sept. 9, 1952. Official Gaz U. S. Pat. Office, **662,** 2: 508. 1952.

Cans of milk in a cabinet are cooled by a spray of cooling water from a distributor pipe located along the necks of the cans. The cans rest on a roller conveyor mounted in the bottom of the cabinet. R. Whitaker

623. Milk cooling attachment for milking machines. E. E. LUNDVALL, R. W. BECKER and C. ECKSTEIN (assignors to Instant Milk Cooling Co., Inc.). U. S. Patent 2,610,034. 13 claims. Sept. 9, 1952. Official Gaz. U. S. Pat. Office, **662,** 2: 508. 1952.

This device cools milk as it enters the reservoir of a milking machine. The cooling is done under vacuum. R. Whitaker

624. Means for milking domestic farm animals and for temporarily storing milk and cooling it. G. R. DUNCAN. U. S. Patent Reissue 23,544. 17 claims. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 57. 1952.

U. S. Patent 2,470,979 (J. Dairy Sci. Abs. #623, 1949) has been revised and improved. R. Whitaker

625. Milking machine. J. A. KINGSTON (assignor to Gascoignes (Reading) Ltd.). U. S. Patent 2,608,951. 4 claims. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 133. 1952.

A complete milking unit is on wheels for moving up an aisle between rows of cows. Provision is made for collecting the milk from each cow, weighing same, cooling it and then delivering it to a storage tank under a controlled amount of vacuum. R. Whitaker

626. Side opening milk cooler. R. D. PUSEY (assignor to Wilson Refrigeration, Inc.). U. S. Patent 2,608,836. 2 claims. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 100. 1952.

Milk cans are inserted in the cabinet through a side door. A 2nd compartment, equipped with cooling coils, contains water which is pumped to sprays circling the necks of the milk cans. The water collects in a pump beneath the cans and is returned to the 2nd compartment for reuse. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

627. Consumers favor high sugar ice cream, research project indicates. Anonymous. Ice Cream Rev., **36,** 1: 116–117. Aug., 1952.

The research committee of the Flavoring Extract Manufacturers Assoc., in collaboration with 6 colleges, recently has completed a study in which it was determined that the sugar content of ice cream plays a more important role than the amount of vanilla used on the taste preferences of consumers.

Four ice cream samples were used for each series of taste tests. Two contained 17% sugar, and the other 2 contained only 11% sugar. One each of the high and low sugar mixes was flavored with 4 oz. of a single-strength pure Bourbon bean vanilla extract/5 gal. of mix, whereas double this amount of vanilla was used in the other 2 mixes. Samples were tasted and ranked by 271 consumers. Results indicated a marked preference for ice cream with higher sugar content (17%), regardless of the level of vanilla. Of the 2 low-sugar samples, the more highly flavored sample was preferred. W. J. Caulfield

628. Pushbutton liquid mixing. H. G. ASSELSTINE and E. MAY, Golden Valley Creamery Co., Newman, Calif. Food Eng., **24,** 8: 71, 157, 159. Aug., 1952.

Planned engineering developed an all-liquid, continuous compact ice cream mix-making process. It involves a minimum of operating and labor cleanup costs. All ingredients, in liquid form, are piped directly to the weigh tank. Flow is controlled by pushbuttons and ingredients are blended by a cycling system. Flow of the mix then is directed to the HTST pasteurizer. After heating to 152° F., the mix is homogenized and returned to the plate unit for pasteurizing. After cooling, the mix is pumped to storage. With the system, a single operator can assemble, blend and pasteurize a variety of ice cream mixes in a continuous operation. T. J. Claydon

629. Hardening tunnels. L. BUEHLER, Creamery Pkg. Mfg. Co., Chicago. Sou. Dairy Prod. J., **51,** 2: 86, 87, 90, 91, 94, 96, 97. Feb., 1952.

The purposes of hardening tunnels are to more quickly harden ice cream and to eliminate the need for forced air circulation in storage rooms. The simplest, most common type consists of a stainless wire-mesh belt 6–12 ft. wide and possibly 40–50 ft. long installed in a passage maintained at −25 to −50° F. with an air-blast system. For a single conveyor, the floor space required for pt. packages is about 1.3 ft.²/gal.; 1–2 hr. are required for hardening. Two or 3 conveyors may be placed 1 above the other to conserve floor space. Other kinds of conveyors which are much more compact may be employed.

Minus 25 rather than −50° F. and a longer hardening time lowers refrigeration requirements and is satisfactory. Contact freezing eliminated 1 heat exchange, eliminated a power blower and passage between 2 contact plates prevents bulging of packages, but only flat-sided packages of 1 size and thickness could be handled in the same equipment. Hardening time varies as the square of the thickness of the package.

Tunnel hardening ties in well with continuous freezing and filling, but ordinarily storage space is required for fluctuations in sales and for different flavors. After hardening, space may be conserved by packing the ice cream solid for storage. Tunnel freezing produces more uniformity in freezing but results in higher refrigeration peak loads as compared with slower freezing.

Booster systems lower the horsepower requirements/ton of refrigeration, lessen pressure differential and lower temperature in the compressor, lower upkeep costs, facilitate reaching low temperatures and increase life of compressors. The original cost of the machinery also is lower. Multicylinder boosters and compressors are more compact than 2-cylinder machines. F. W. Bennett

630. Frozen custard machine. B. H. WOODRUFF. U. S. Patent 2,608,833. 8 claims. Sept. 2, 1952. Official Gaz. U. S. Pat. Office, **662,** 1: 99. 1952.

A freezer of the conventional type is built into a refrigerated cabinet where space is provided for

storing the frozen custard, ice cream, ice milk, etc. R. Whitaker

631. California decision paves way for Yogurt sherbet. Anonymous. Ice Cream Rev., **36,** 1: 74. Aug., 1952.

The California Dept. of Agr. recently has defined Yogurt sherbet as a product meeting all the requirements and standards of the agricultural code pertaining to sherbets, except that it must have an acidity of not less than 0.60% calculated as lactic acid and that the bacterial standard for sherbet will not apply to this product. It is anticipated that sale of Yogurt sherbet will now be effectively promoted with the passage of this definition which legalizes its production. W. J. Caulfield

632. Packaging half gallons automatically. W. E. GUNNERSON, Anderson Bros. Mfg. Co., Rockford, Ill. Ice Cream Rev., **36,** 1: 44–45, 86. Aug., 1952.

Production of half gallons of ice cream has been increased from 700 to 1,800 units/hr. through introduction of automatic packaging equipment at the Page Dairy Co., Toledo, O. This has been accomplished with a reduction in personnel engaged in this operation from 6 down to a three-man crew. In addition to increased production the automatic packaging equipment has resulted in a uniformity of fill that was impossible when hand filling was used. W. J. Caulfield

633. New outlets for bulk gallonage when liquor and ice cream get together. Anonymous. Ice Cream Rev., **36,** 1: 46–47, 58–59. Aug., 1952.

An undeveloped market for ice cream has been uncovered by the Evergreens Restaurant at Dundee, Ill., in the form of a liquor ice. In producing this item approximately 6 oz. of New York ice cream are placed in a mixing cup to which 0.75 oz. of the customers' favorite liquor is added. After mixing on a mixer for 2–3 min., the tasty beverage dessert is poured into a chilled silver dessert dish and served with a straw.

This drink has proved to be a popular item on the desert menu; potential possibilities as an outlet for ice cream are indicated by the fact that 18 gal. of ice cream were used in preparing this drink in 1 wk. during the summer of 1951. W. J. Caulfield

634. Machine for drying and bagging frozen confections. E. TUMUSKO. U. S. Patent 2,608,329. 2 claims. Aug. 26, 1952. Official Gaz. U. S. Pat. Office, **661,** 4: 1048. 1952.

The chocolate coating of frozen confections is dried rapidly by a stream of air prior to dropping into air-inflated bags. R. Whitaker

635. A sanitation study of fountain mixed milk drinks. F. H. FISKE, Depts. of Health and Hospitals, Denver, Col. J. Milk & Food Technol., **15:** 167–174. July–Aug., 1952.

Examination of 119 chocolate milk shakes as prepared at the fountain indicated that 25% were below standard for butterfat. One-half of the samples were above standard in bacterial quality. The chocolate syrup showed excessive bacterial counts in 34% of the samples. Also, use of sub-standard constituents in the final product was evident. Poor sanitization of soda fountain utensils, especially the ice cream dipper, was observed. H. H. Weiser

636. Vegetable oils in frozen desserts. E. M. DECK, Mrs. Tucker's Foods, Inc., Sherman, Texas. Ice Cream Rev., **35,** 12: 45–47, 74–77. July, 1952.

A hydrogenated vegetable fat with a melting point of 106–114° F. without the use of an emulsifier proved most satisfactory for the production of a vegetable-fat frozen dessert. The manufacturing process in the production of this type of frozen dessert is essentially the same as for ice cream. The author recommends a mix containing 10–12% vegetable fat and an overrun of 80–90%.

Formulas are presented for a series of mixes ranging from 2.5–14% fat, compounded from condensed skimmilk, vegetable fat, sugar, corn syrup solids, stabilizer and water.

The importance of not misrepresenting vegetable-fat frozen desserts to the consumer is stressed. W. J. Caulfield

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

637. Pasteurizer. H. B. SAUNDERS (assignor to Guard-It Manufacturing Co.). U. S. Patent 2,607,566. 4 claims. Aug. 19, 1952. Official Gaz. U. S. Pat. Office, **661,** 3: 771. 1952.

An electrically heated, water-jacketed, mechanically agitated small pasteurizer for home use is described. R. Whitaker

638. Waxes suitable for various types of paper milk containers. C. SWETT, Natl. Wax Co., Chicago, Ill. Milk Dealer, **41,** 10: 90–93. July, 1952.

Glue is important but the extent of resistance of the finished containers to liquid depends on the wax. Microcrystalline wax is used as a modifier of the paraffin used on the paper milk container. It is tougher and more flexible than paraffin and even on the lower melting point paraffin of 125–127° F. use of 2–3% helps to reduce leakers by strengthening the paraffin and increasing its bonds to the paper. It is particularly important to use an increasing percentage of microcrystalline wax when paraffins of higher melting points are used. The recommended percentages are 2–3% for 125–127°, 3–4% for 128–130° and 4–5% for 130–132°. These microcrystalline waxes can be added at the dairy. Several producers, however, supply dairy waxes of paraffin already blended with the proper amount of microcrystalline wax. Use of too high a percentage of the wax may result in excessive bulging of the carton. C. J. Babcock

639. Bottle closure. R. SONNENBERG (assignor to Mid-West Bottle Cap Co.). U. S. Patent 2,607,503. 12 claims. Aug. 19, 1952. Official Gaz. U. S. Pat. Office, **661,** 3: 753. 1952.

A metal foil milk bottle cap which covers the bead and extends to the cap seat is held in place by a disc pressed into the cap seat. This disc expands slightly when wet. A paraffin wax-impregnated liner is laminated to the foil. Heat applied when the cap is formed melts the wax, which penetrates the folds of the crimped foil.
R. Whitaker

640. Bottle closure. R. SONNENBERG (assignor to Mid-West Bottle Cap Co.). U. S. Patent 2,607,504. 2 claims. Aug. 19, 1952. Official Gaz. U. S. Pat. Office, **661,** 3: 753. 1952.

A partially preformed cover-all milk bottle closure, consisting of a layer of paper laminated to metal foil with micro-crystalline wax, is described. Letters and designs, depressed into the foil, are colored with ink at the bottom of the depressions. R. Whitaker

641. Container with flat top and integral overcap. G. C. REID and S. S. JACOBS (assignors to American Can Co.). U. S. Patent 2,609,985. 3 claims. Sept. 9, 1952. Official Gaz. U. S. Pat. Office, **662,** 2: 493. 1952.

A paper flap, hinged on a diagonal line drawn across the top of a Canco type of paper milk bottle, provides protection for the pouring lip and bottle closure. R. Whitaker

642. Milk bottle holder. E. M. NAVARRO. U. S. Patent 2,609,987. 1 claim. Sept. 9, 1952. Official Gaz. U. S. Pat. Office, **662,** 2: 493. 1952.

A bracket for holding and locking milk bottles on consumers' doorsteps is described.
R. Whitaker

643. Refrigerant attachment for cartons. G. D. WHEELER and J. H. REILLY. U. S. Patent 2,609,670. 2 claims. Sept. 9, 1952. Official Gaz. U. S. Pat. Office, **662,** 2: 401. 1952.

Bottled liquids, such as milk in cases or cartons, are maintained under refrigeration by use of this device, which consists of blocks of refrigerants attached to the inside surface of the top of the carton. R. Whitaker

644. Flavor and keeping quality of vitaminized fortified skimmilk. K. G. WECKEL, Univ. of Wis., Madison. Milk dealer, **41,** 11: 56–60. Aug., 1952.

The production of excellent low-fat vitamin-fortified modified skimmilk products requires selection of high quality ingredients. Recommended practices include the addition of 0.5–0.75% fat and an increase of 1.0–3.0% in the solids-not-fat. Homogenization of low-fat milk in which either or both fat or solids-not-fat have been added results in improved flavor and avoids potential solids sedimentation. Temperature ranges of from 150–175° F. for fat processing and 168–180° F. for HTST operations are most suitable. Use of brown glass bottles and wax paper cartons have been about equally effective in minimizing the destructive effect of the radiation on the vitamins. These products are subject to aging and spoilage effects as are other dairy products and must be handled accordingly.
C. J. Babcock

645. Incentive for automatic milk vending machines. R. H. BUNDE, Land O' Lakes Creameries, Inc., Minneapolis, Minn. Milk Dealer, **41,** 10: 42–43, 67–74. July, 1952.

Experience with 7 automatic vendors in the surburban area of Minnesota's Twin Cities by the St. Michael Cooperative Assoc. has shown that the machine must maintain a uniform temperature of 32–36° F. winter and summer, meet local sanitation codes, lend itself to ease of cleaning and have a storage capacity large enough to minimize delivery expenses and insure against shortage. A location which is open 24 hr. a day, orderly and well lighted at night, is an asset. A town or area with a minimum population of 4,000 within 8 blocks usually is a good location. Transient traffic alone will not justify a large-volume machine. Break-even point is about 200 qt. or 100 0.5-gal. containers a day. A table shows a cost analysis of the 7 automats. Cost for the entire month of April, 1952, was $.026354/qt. from the plant to consumer. Increased sales volumes during the 1st week of May reduced this to $.019699. Interest, insurance and personal property taxes were not a part of these costs. These are estimated at approximately $11.00/mo./machine. C. J. Babcock

646. Gegenwärtiger Stand der Milchwirtschaft in Brasilien. I. Die Milchversorgung von Rio de Janeiro (Present conditions of dairying in Brazil. I. The milk supply for Rio de Janeiro). J. MINUT. Milchwissenschaft, **6,** 1: 10–12. Jan., 1951.

Rio de Janeiro, with a population of about 2.5 million, consumes about 280–290,000 l. of fluid milk daily. This is produced in 3 provinces on approximately 4,500 farms of various sizes. Most milk is pasteurized at high temperature, while some is sold raw. Some milk is bottled and delivered to the home; some is measured out on the street into diverse types of kitchen utensils from large stainless steel or aluminum tank trucks. The composition requirements for fluid milk are: 3% fat, 8.5% S.N.F., 11.5% T-S, 4.3% lactose and 15–20° D. acid. I. Peters

647. Über aktuelle Meiereiforschungs-Aufgaben in Schweden (Important dairy research problems in Sweden). English summary. K. E. THOMÉ and P. SWARTLING. Milchwissenschaft, **6,** 1: 3–6. Jan., 1951.

The annual production of milk in Sweden is slightly higher than its domestic demand and amounts to approximately 4,500,000 tons of which 900,000 tons are used as fluid milk, 2,000,000 tons for butter and 600,000 tons for cheese. Research concerned on these products is carried out at the National Dairy Research Station at Alnarp. Work on practical problems is done at Örnsköldsvic, except on powdered milk which is done by the industry.

Problems concerned with improved methods of milk production involve use of silage, as well as farm and plant hygiene. Main problems with butter involve oxidized flavor and continuous buttermaking; with cheese the use of pasteurized

milk, microbiological studies of cheese ripening and mold inhibitors suitable for external application alone or in combination with paraffin.

I. Peters

648. The effects of applied electrical potential on oxidized flavor in milk. R. W. Hunt and D. J. Hankinson, Univ. of Mass., Amherst. Sou. Dairy Prod. J., **51,** 2: 122–123, 127. Feb., 1952.

Milk was subjected to direct electrical currents of less than 140 milliamp. and voltages ranging from 0–240 by passing it between electrodes of carbon, tin, nickel, chromium and stainless steel. No significant effects were observed on development of oxidized flavor in milk containing added Cu. A direct current of 6 v. and 6 amp. caused an excessive curd deposit on the electrodes. Severe pitting of the tin electrode occurred in all cases. The electric current apparently caused no new off-flavor or changes in titratable acidity, pH or O-R potential of the milk.

F. W. Bennett

MILK SECRETION

V. R. SMITH, SECTION EDITOR

649. Synthesis of milk fat from acetic acid ($CH_3{}^{14}COOH$) by the perfused isolated bovine udder. A. T. Cowie, W. G. Duncombe, S. J. Folley, T. H. French and R. F. Glascock, Natl. Inst. for Research in Dairying, Reading; L. Massart and G. J. Peeters, Univ. of Ghent, Belgium; and G. Popjak, Natl. Inst. for Med. Research, London. Biochem. J., **49,** 5: 610–615. 1951.

An isolated bovine udder, arranged so each half could be separately perfused simultaneously, was used in this investigation. Approximately 5 l. of heparinized cow's blood were used as the perfusion fluid in each half. To one-half was added $CH_3{}^{14}COONa$ and to the other $NaH^{14}CO_3$ and heavy water (D_2O). The milk secreted from each previously emptied half and the udder tissue were fractionated and analyzed. During the 2-hr. perfusion period, only 140 ml. of milk were secreted from the "acetate" half, while the "bicarbonate" half secreted 1,420 ml. The metabolites furnished by the perfusion blood, especially in the case of the "bicarbonate" half, were much less than that required for the synthesis of the milk fat. Presumably, the difference originated from the udder tissue itself. Fatty acids synthesized in the udder cells apparently were the direct precursors of fatty acids in milk fat. Pronounced decrease in the radioactivity of the fatty acids as the chain length increased occurred, indicating that short-chain acids could not have originated from a degradation of the long-chain acids. The specific activity of the butyric acid was more than 1,000-fold greater than that for oleic acid. Further work showed that cholesterol was synthesized in the udder. Lactose from the "acetate" half was appreciably radioactive, while that from the "bicarbonate" half was not.

A. O. Call

650. Further observations on the in vitro stimulation by insulin of fat synthesis by lactating mammary gland slices. J. H. Balmain and S. J. Folley, Natl. Inst. for Research in Dairying, Reading. Biochem. J., **49,** 5: 663–670. 1951.

Respiratory measurements on lactating rat mammary gland slices were stimulated by addition of insulin when glucose plus acetate were present. Slight increases from insulin were noted when glucose alone was present but none when acetate alone was present. This response to insulin was not noted when the mammary slices came from rats at the end of pregnancy or after weaning. Lactating rabbit mammary tissue acted similarly to rat mammary tissue but mouse mammary tissue was less responsive to insulin. Udder slices from lactating ewes showed no response. Added glycerol acted similarly to added insulin on lactating rat mammary tissue, but addition of glycerol and insulin was not additive in effect.

A. O. Call

651. The effect of thyroxine and thiouracil on the composition of milk. I. The partition of phosphorus in cow milk in relation to phosphatase and other constituents. R. Chanda and E. C. Owen, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **50,** 1: 100–109. 1951.

Eight cows in mid-lactation were used in 2 experiments—3 in the first and 5 in the second. The effect of injections of thyroxine and thiouracil upon the P fractions and upon the N of the milk was measured. Cows required about twice as much thiouracil as thyroxine to give a milk phosphatase increase equivalent to the decrease due to thyroxine. Neither treatment changed significantly the N partition in the casein, albumin, globulin or non-protein nitrogen fractions, but thyroxine increased and thiouracil decreased the P content of the milk. Ester and lipid P were particularly increased by thyroxine. The increase in metabolic rate brought about by thyroxine may increase the phosphorylating enzymes. Thyroid activity may influence the P content of casein.

A. O. Call

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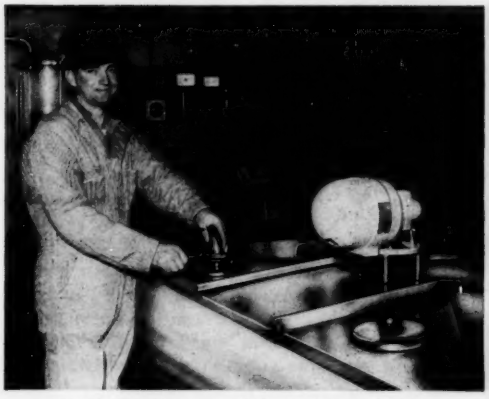

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